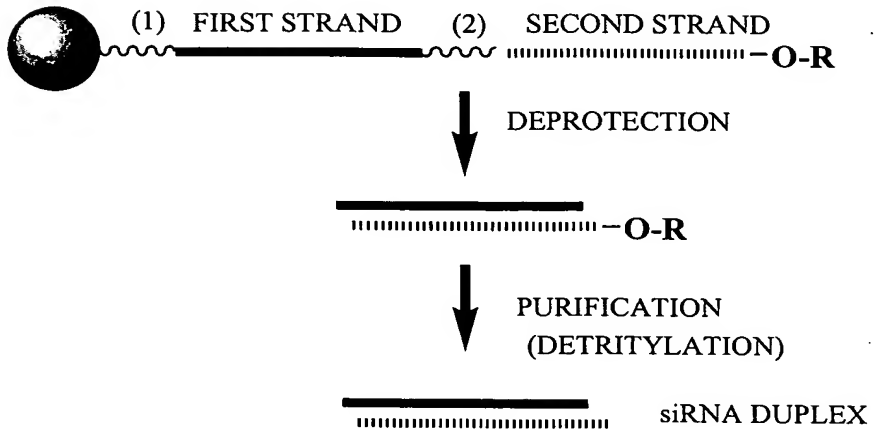
Figure 1

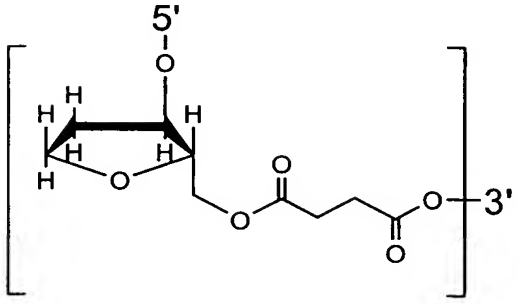


= SOLID SUPPORT

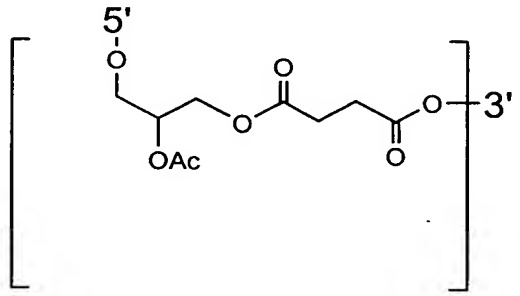
R = TERMINAL PROTECTING GROUP
FOR EXAMPLE:
DIMETHOXYTRITYL (DMT)

(1) = CLEAVABLE LINKER
(FOR EXAMPLE: NUCLEOTIDE SUCCINATE OR INVERTED DEOXYABASIC SUCCINATE)

(2) = CLEAVABLE LINKER
(FOR EXAMPLE: NUCLEOTIDE SUCCINATE OR INVERTED DEOXYABASIC SUCCINATE)



INVERTED DEOXYABASIC SUCCINATE LINKAGE



GLYCERYL SUCCINATE LINKAGE

Figure 2

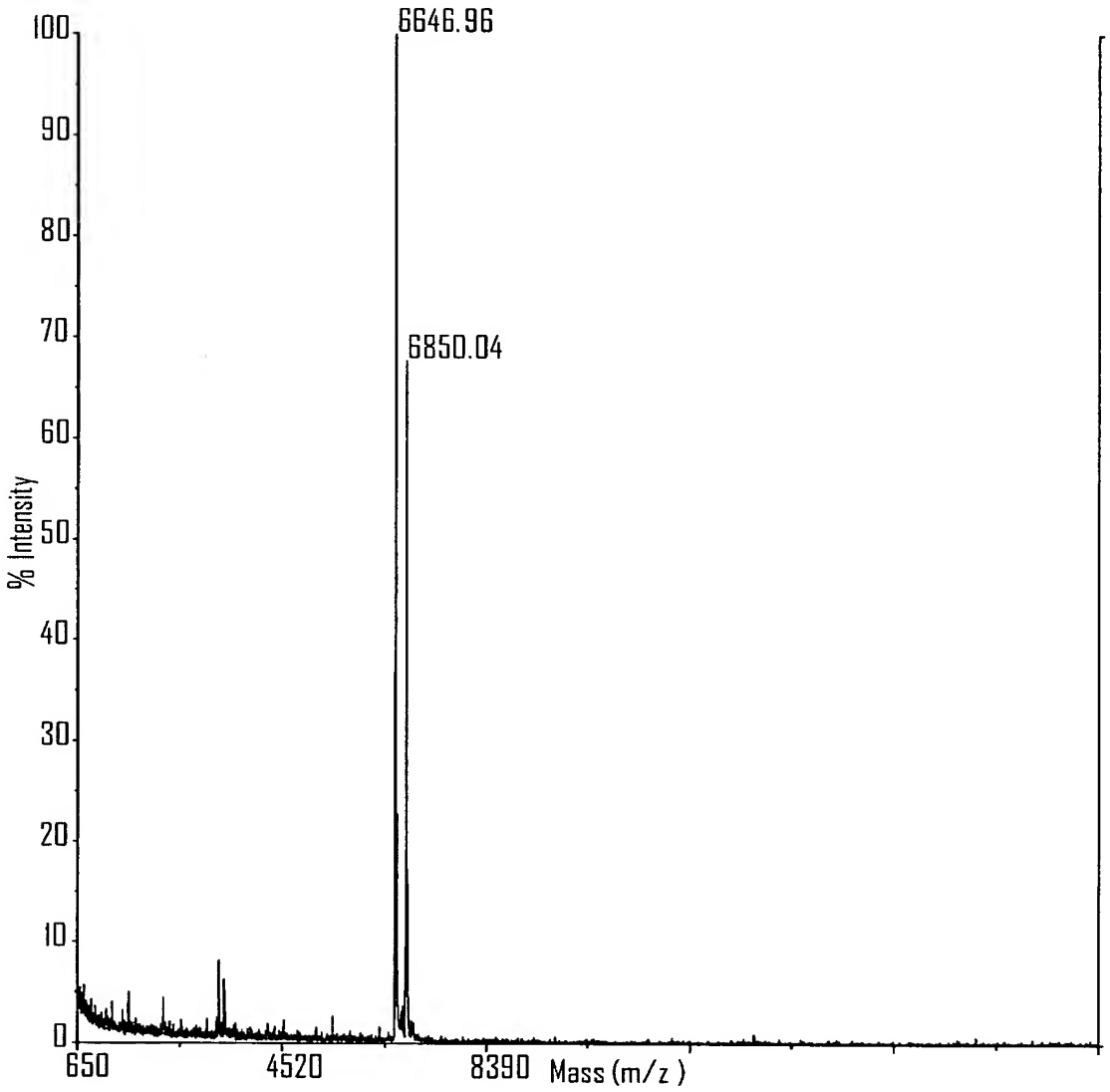
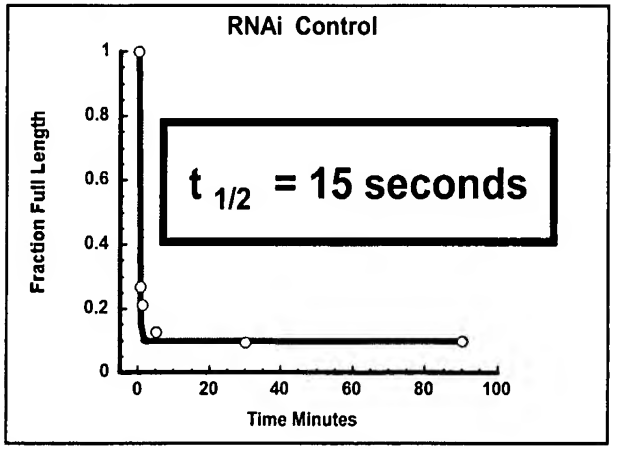

6646.96
6850.04
100
90
80
70
60
50
40
30
20
10
0
% Intensity
650
4520
8390
Mass (m/z)

Figure 3

5'-CGUACGCGGAAUACUUCGATT (SEQ ID NO: 394) 3'-TTGCAUGCGCCUUAUGAAGCU (SEQ ID NO: 395)	T ½ = 15 seconds (control)
5'-B cAAccAcAAAAuAcAAcAATT B (SEQ ID NO: 396) 3'-TXGuuGGuGuuuuAuGuuGuu (SEQ ID NO: 397)	T ½ = 138 min
5'-B cAAccAcAAAAuAcAAcAATT B (SEQ ID NO: 396) 3'-TDGuuGGuGuuuuAuGuuGuu (SEQ ID NO: 398)	T ½ = 3.7 days
5'-B cAAccAcAAAAuAcAAcAATT B (SEQ ID NO: 396) 3'-XTGuuGGuGuuuuAuGuuGuu (SEQ ID NO: 399)	T ½ = 72 minutes
5'-B cAAccAcAAAAuAcAAcAATT B (SEQ ID NO: 396) 3'-LTGuuGGuGuuuuAuGuuGuu (SEQ ID NO: 400)	T ½ = 40 days
5'-B cAAccAcAAAAuAcAAcAATT B (SEQ ID NO: 396) 3'-tTGuuGGuGuuuuAuGuuGuu (SEQ ID NO: 401)	T ½ = 32 days

G, A, U, C = Guanosine, Adenosine, Uridine, Cytidine
T = Thymidine
Lower Case = 2'-deoxy-2'-fluoro
S = phosphorothioate
B = inverted deoxyabasic
D = inverted Thymidine
X = 3'-deoxy Thymidine
t = L-thymidine
L = Glyceryl moiety



Title: RNA Interference Mediated Inhibition of Gene Expression Using Chemically Modified Short Interference Nucleic Acid (siNA)
Inventors: McSwiggen et al.
Attorney Docket No. 03-465-D(400.151)
Sheet 3 of 113

Figure 4

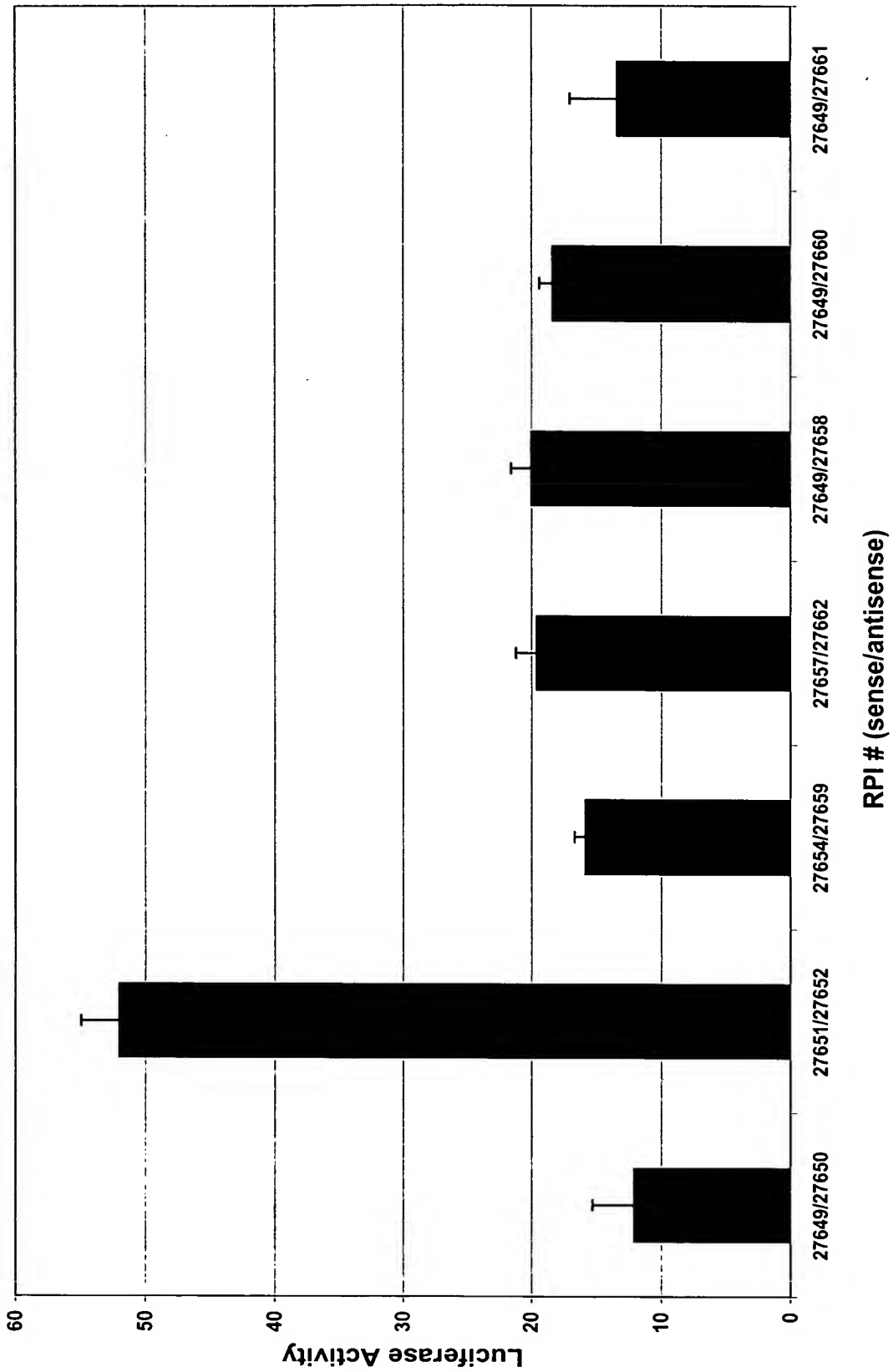

Luciferase Activity
0
10
20
30
40
50
60
27649/27650
27651/27652
27654/27659
27657/27662
27649/27658
27649/27660
27649/27661
RPI # (sense/antisense)

Figure 5

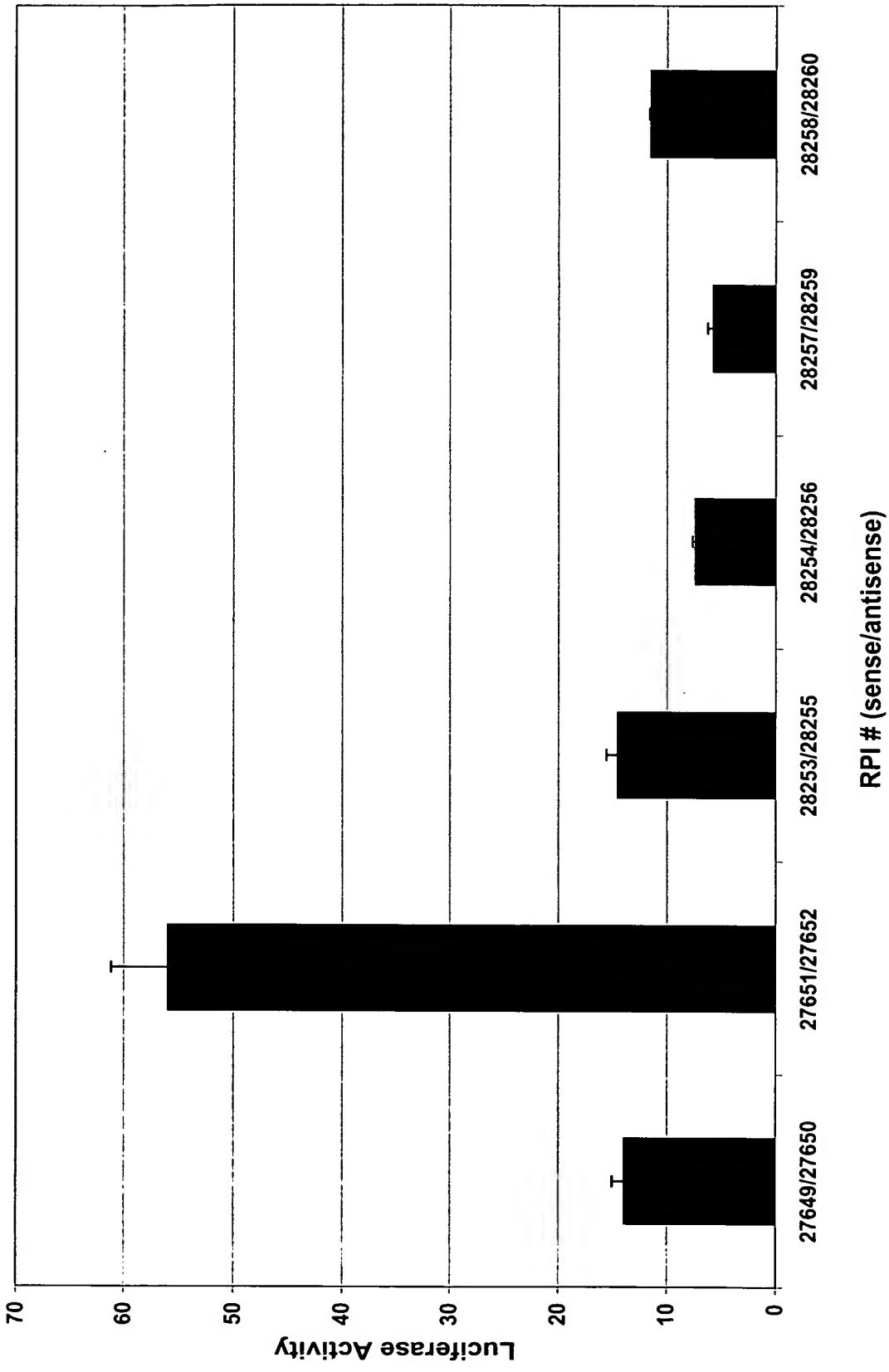

Luciferase Activity
70
60
50
40
30
20
10
0
27649/27650
27651/27652
28253/28255
28254/28256
28257/28259
28258/28260
RPI # (sense/antisense)

Figure 6

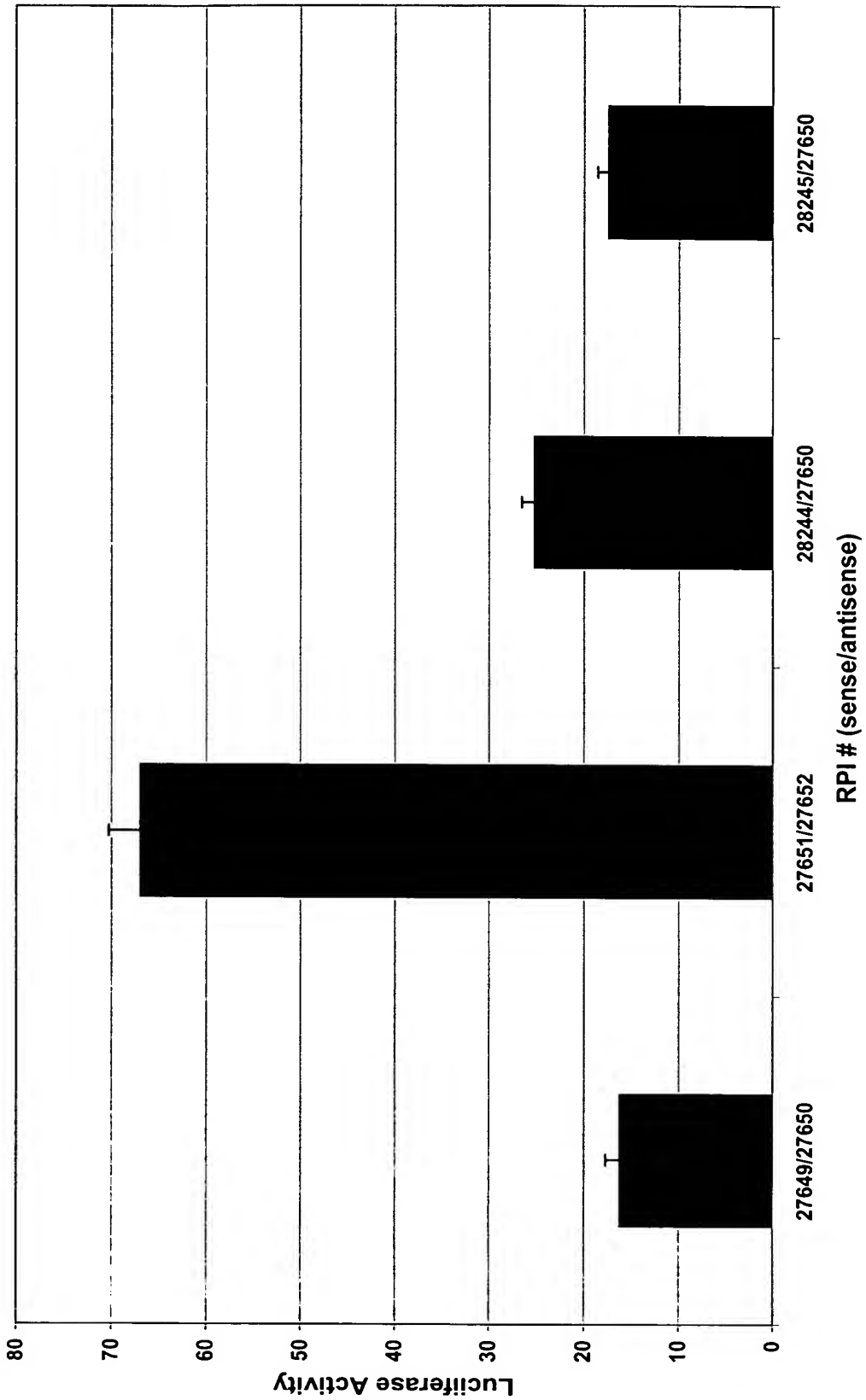

Luciferase Activity
80
70
60
50
40
30
20
10
0
27649/27650
27651/27652
28244/27650
28245/27650
RPI # (sense/antisense)

Figure 7

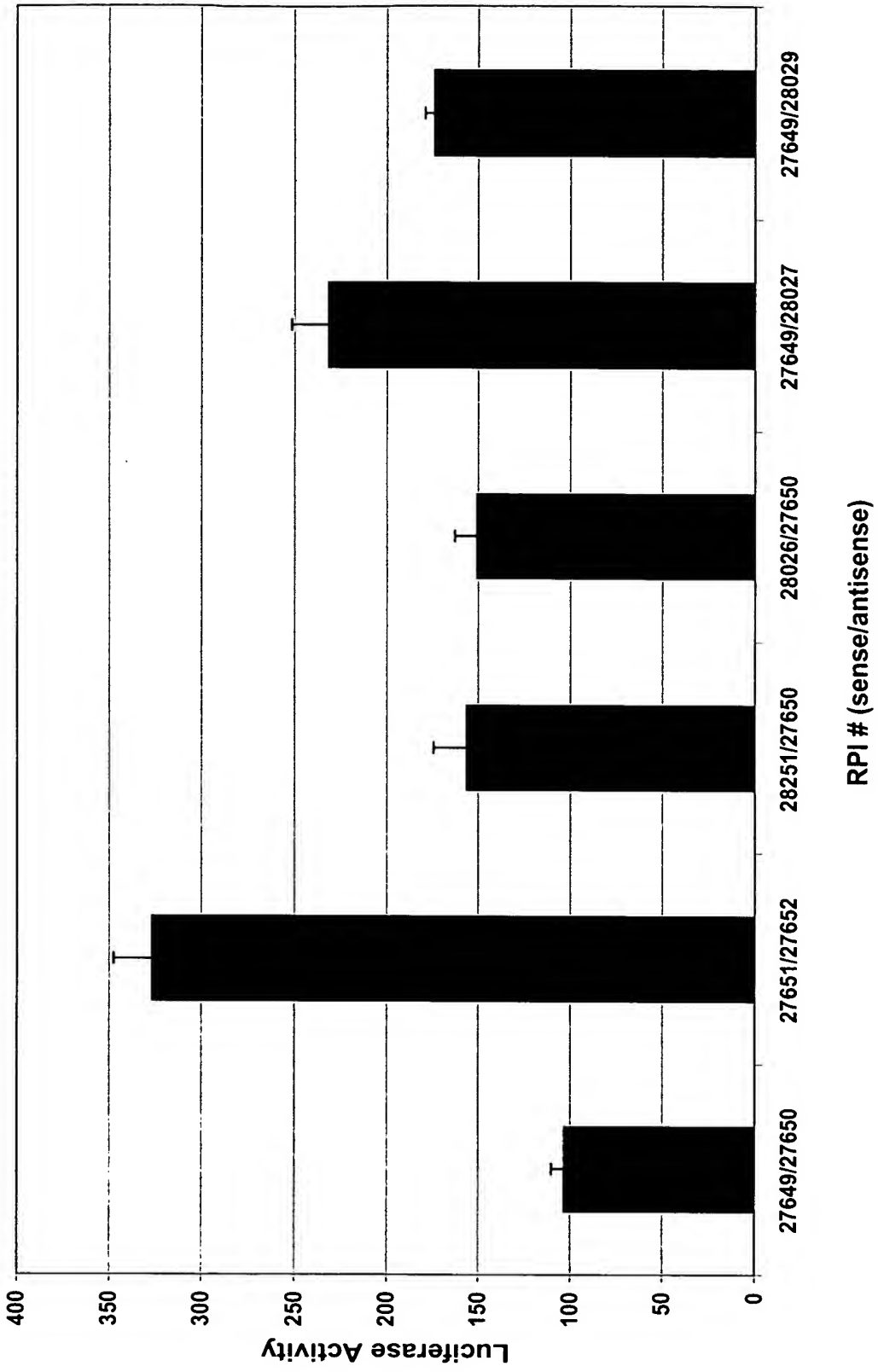

Luciferase Activity
400
350
300
250
200
150
100
50
0
27649/27650
27651/27652
28251/27650
28026/27650
27649/28027
27649/28029
RPI # (sense/antisense)

Figure 8

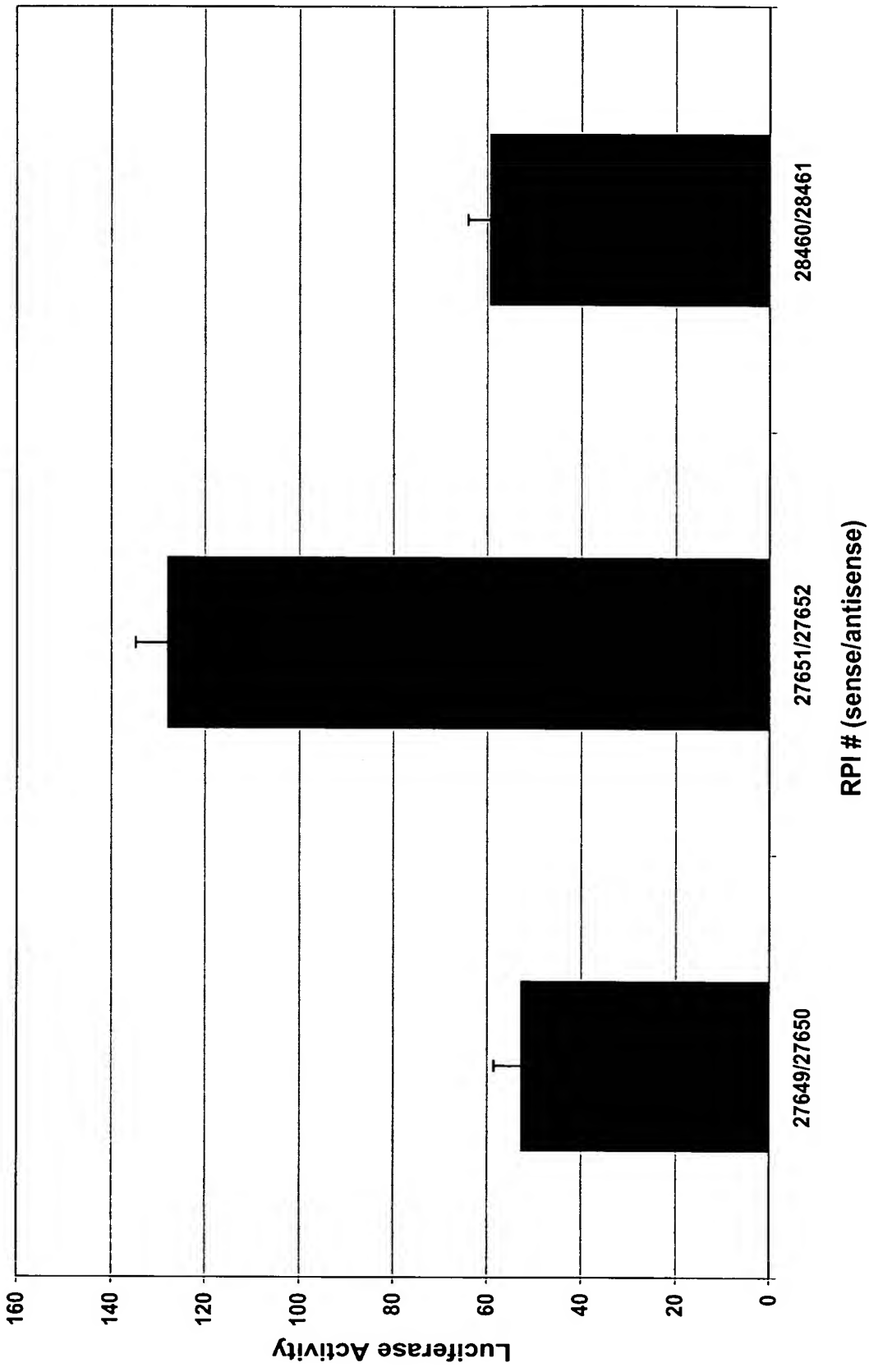

Luciferase Activity
160
140
120
100
80
60
40
20
0
27649/27650
27651/27652
28460/28461
RPI # (sense/antisense)

Figure 9

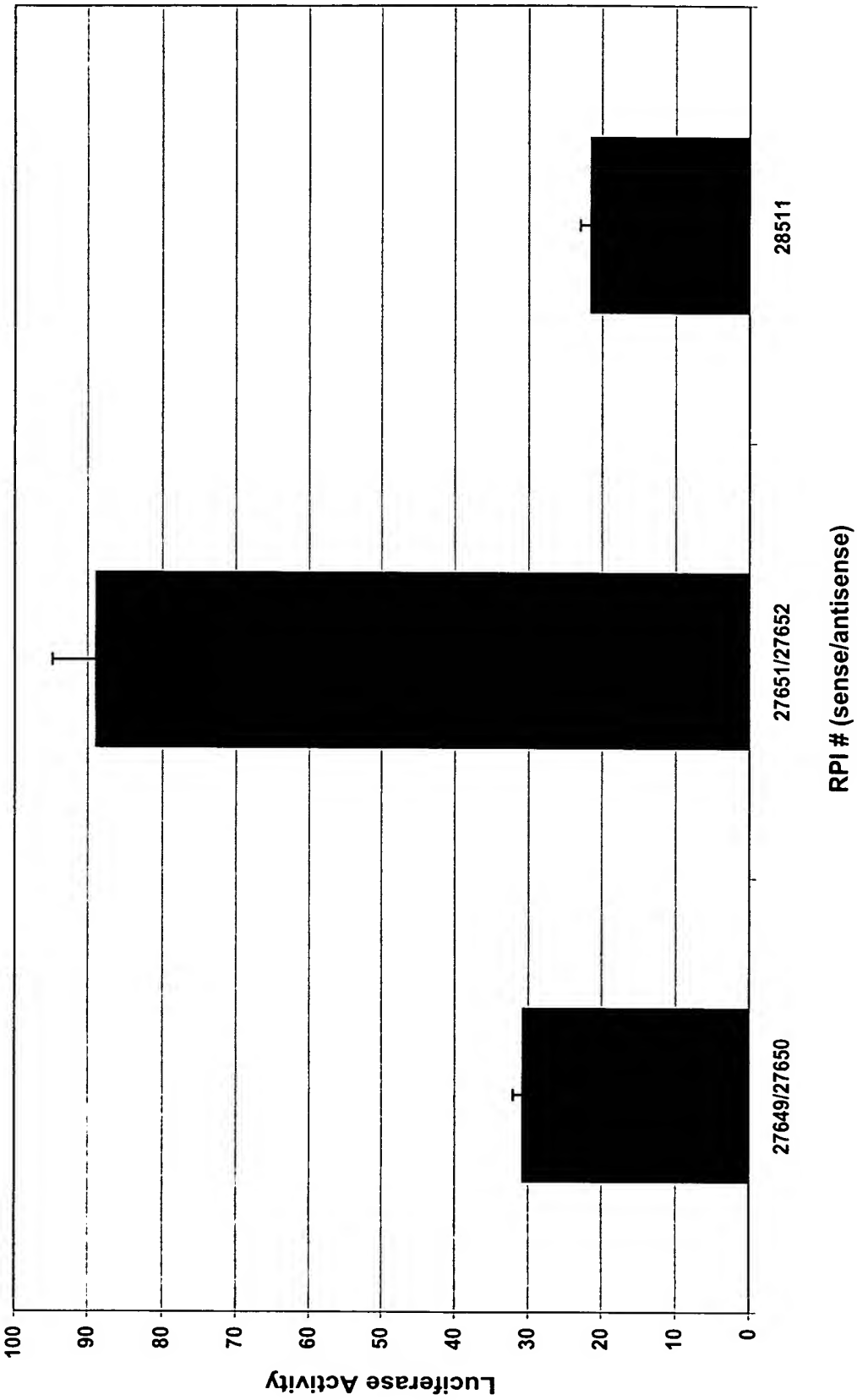

Luciferase Activity
100
90
80
70
60
50
40
30
20
10
0
27649/27650
27651/27652
28511
RPI # (sense/antisense)

Figure 10

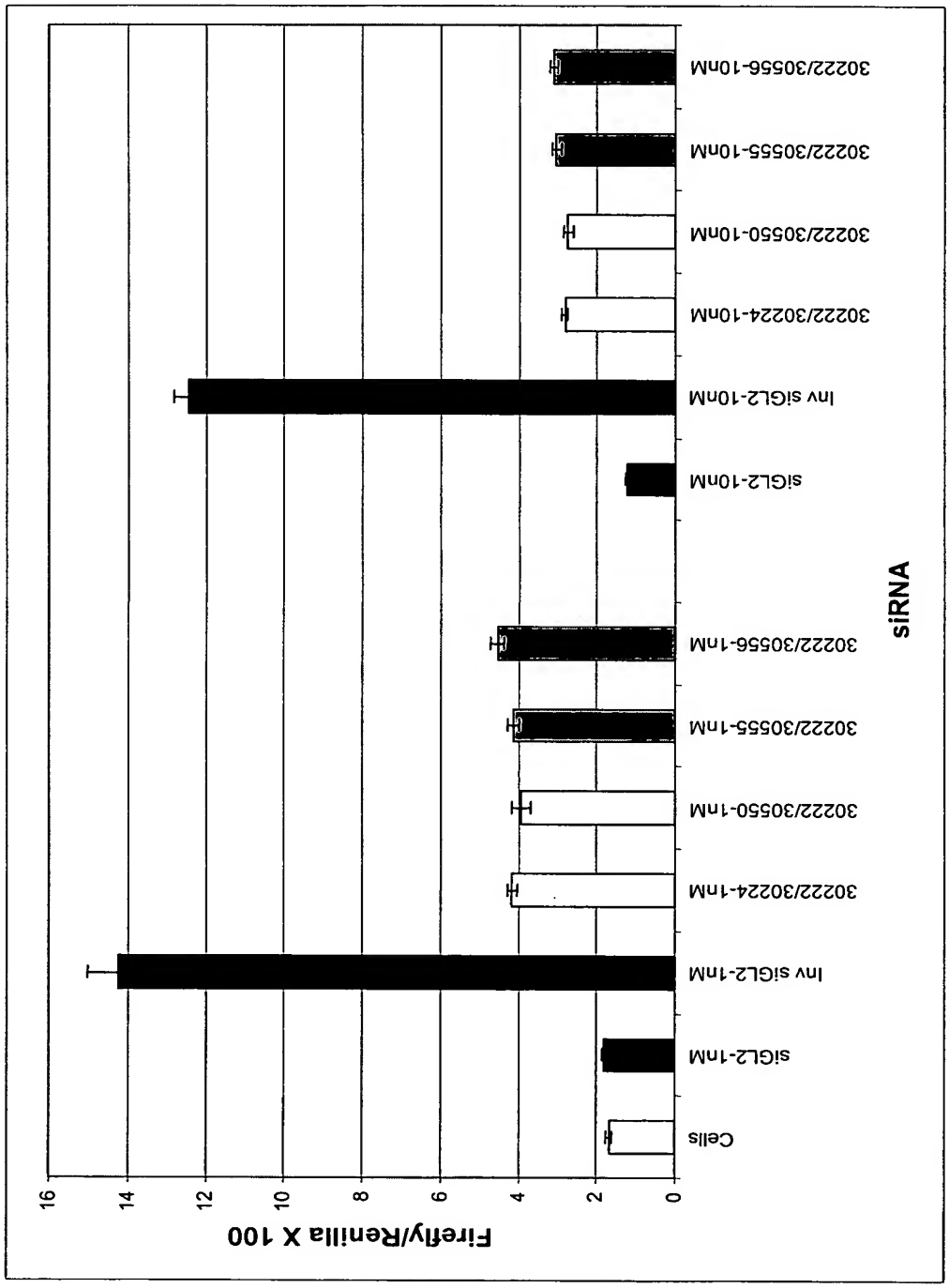

Firefly/Renilla X 100
0
2
4
6
8
10
12
14
16
siRNA
Cells
siGL2-1nM
Inv siGL2-1nM
30222/30224-1nM
30222/30550-1nM
30222/30555-1nM
30222/30556-1nM
siGL2-10nM
Inv siGL2-10nM
30222/30224-10nM
30222/30550-10nM
30222/30555-10nM
30222/30556-10nM

Figure 11

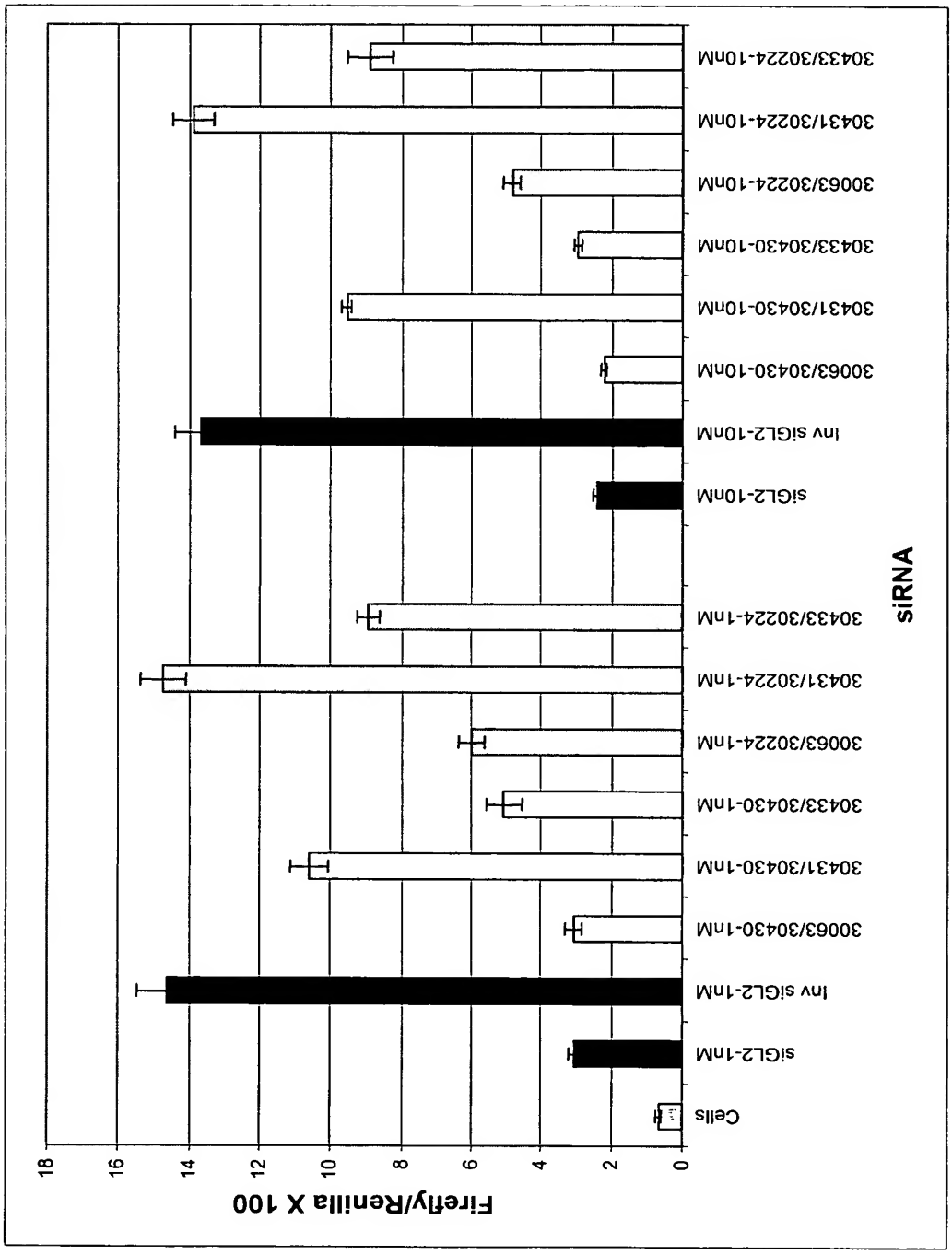

Firefly/Renilla X 100
0
2
4
6
8
10
12
14
16
18
siRNA
Cells
siGL2-1nM
Inv siGL2-1nM
30063/30430-1nM
30431/30430-1nM
30433/30430-1nM
30063/30224-1nM
30431/30224-1nM
30433/30224-1nM
siGL2-10nM
Inv siGL2-10nM
30063/30430-10nM
30431/30430-10nM
30433/30430-10nM
30063/30224-10nM
30431/30224-10nM
30433/30224-10nM

Figure 12

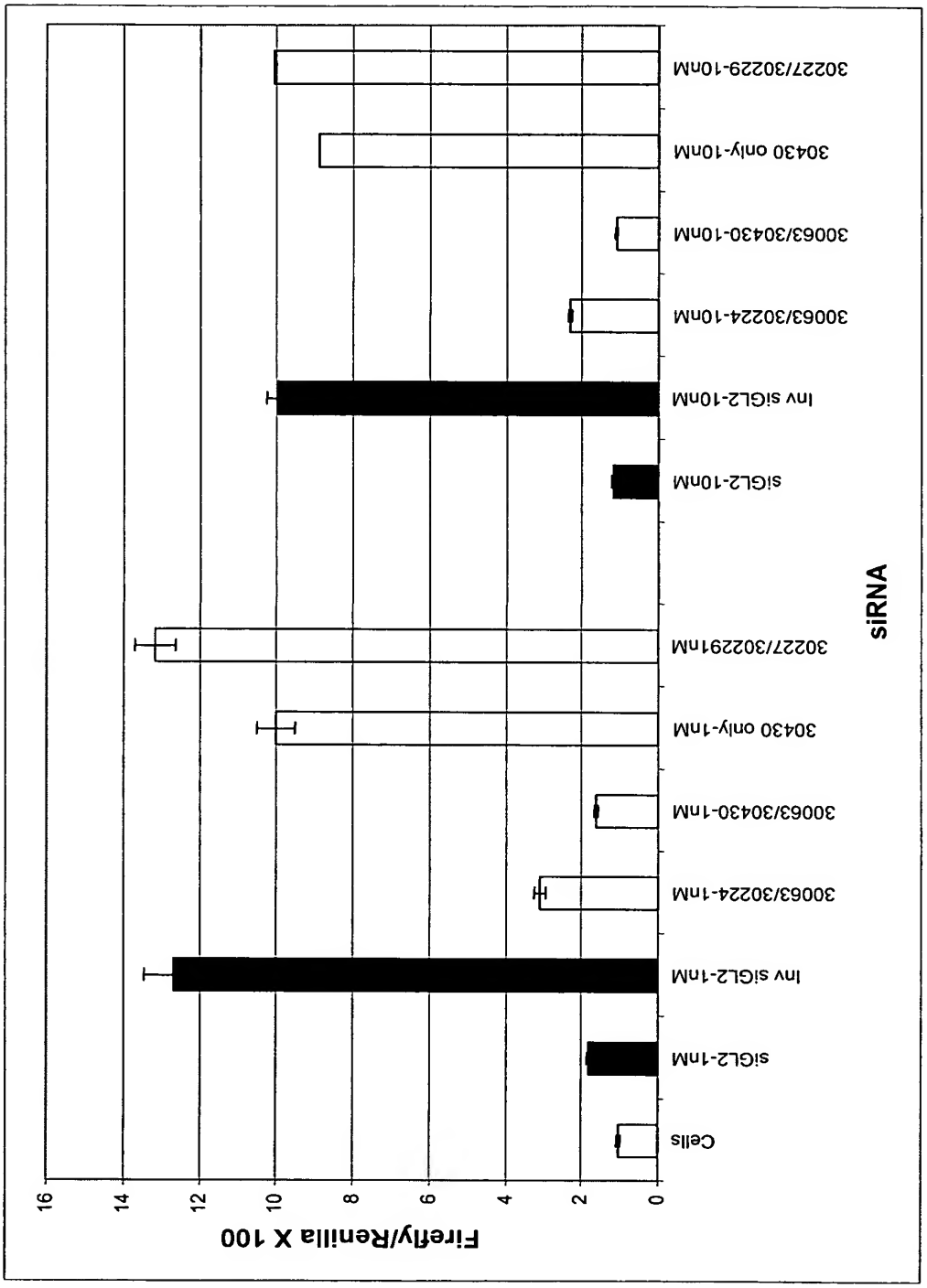

Firefly/Renilla X 100
0
2
4
6
8
10
12
14
16
siRNA
Cells
siGL2-10nM
Inv siGL2-10nM
30063/30224-10nM
30063/30430-10nM
30430 only-10nM
30227/30229-10nM
30227/30229-1nM
30430 only-1nM
30063/30430-1nM
30063/30224-1nM
Inv siGL2-1nM
siGL2-1nM

Figure 13

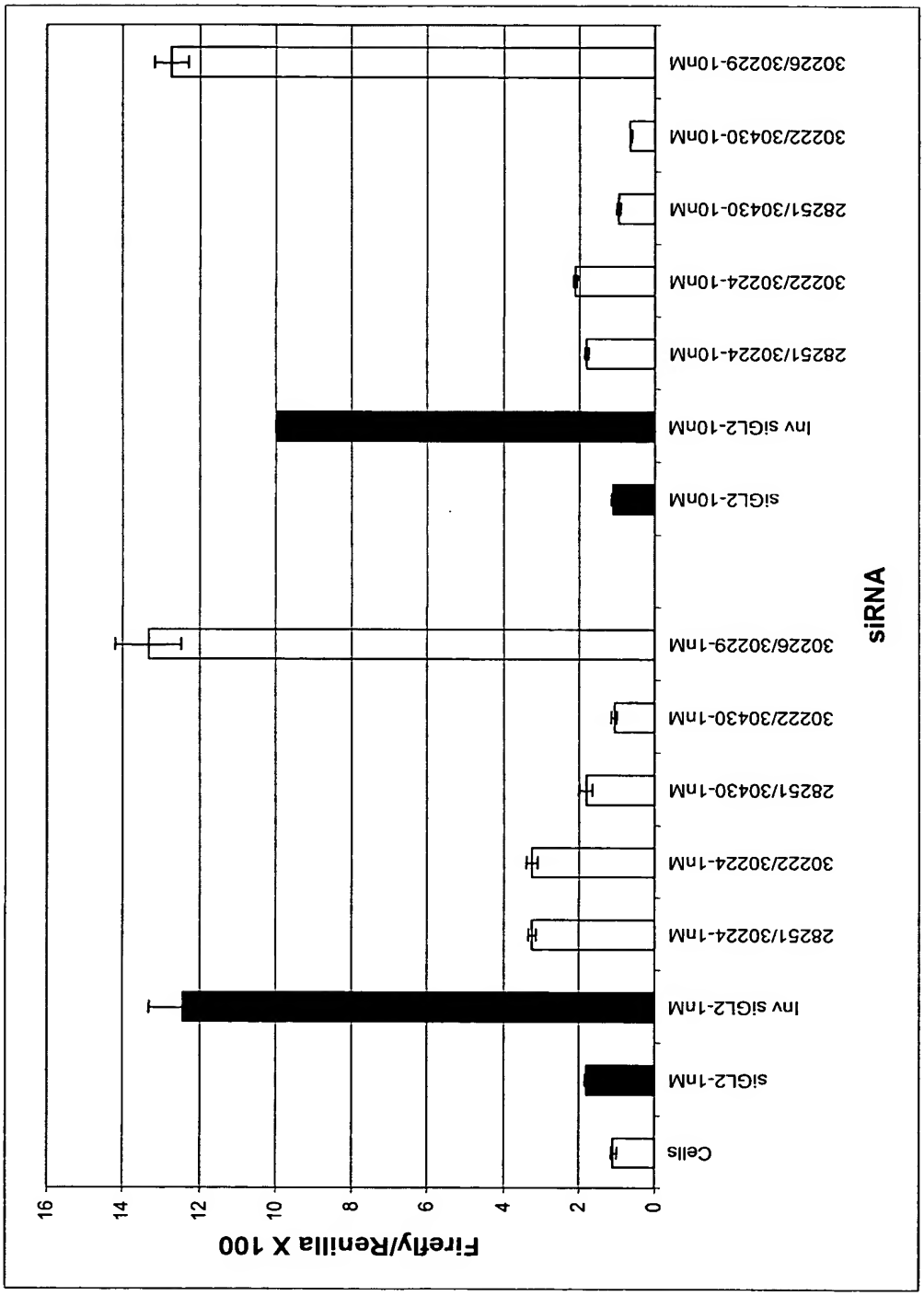

Firefly/Renilla X 100
0
2
4
6
8
10
12
14
16
siRNA
Cells
siGL2-1nM
Inv siGL2-1nM
28251/30224-1nM
30222/30224-1nM
28251/30430-1nM
30222/30430-1nM
30226/30229-1nM
siGL2-10nM
Inv siGL2-10nM
28251/30224-10nM
30222/30224-10nM
28251/30430-10nM
30222/30430-10nM
30226/30229-10nM

Figure 14

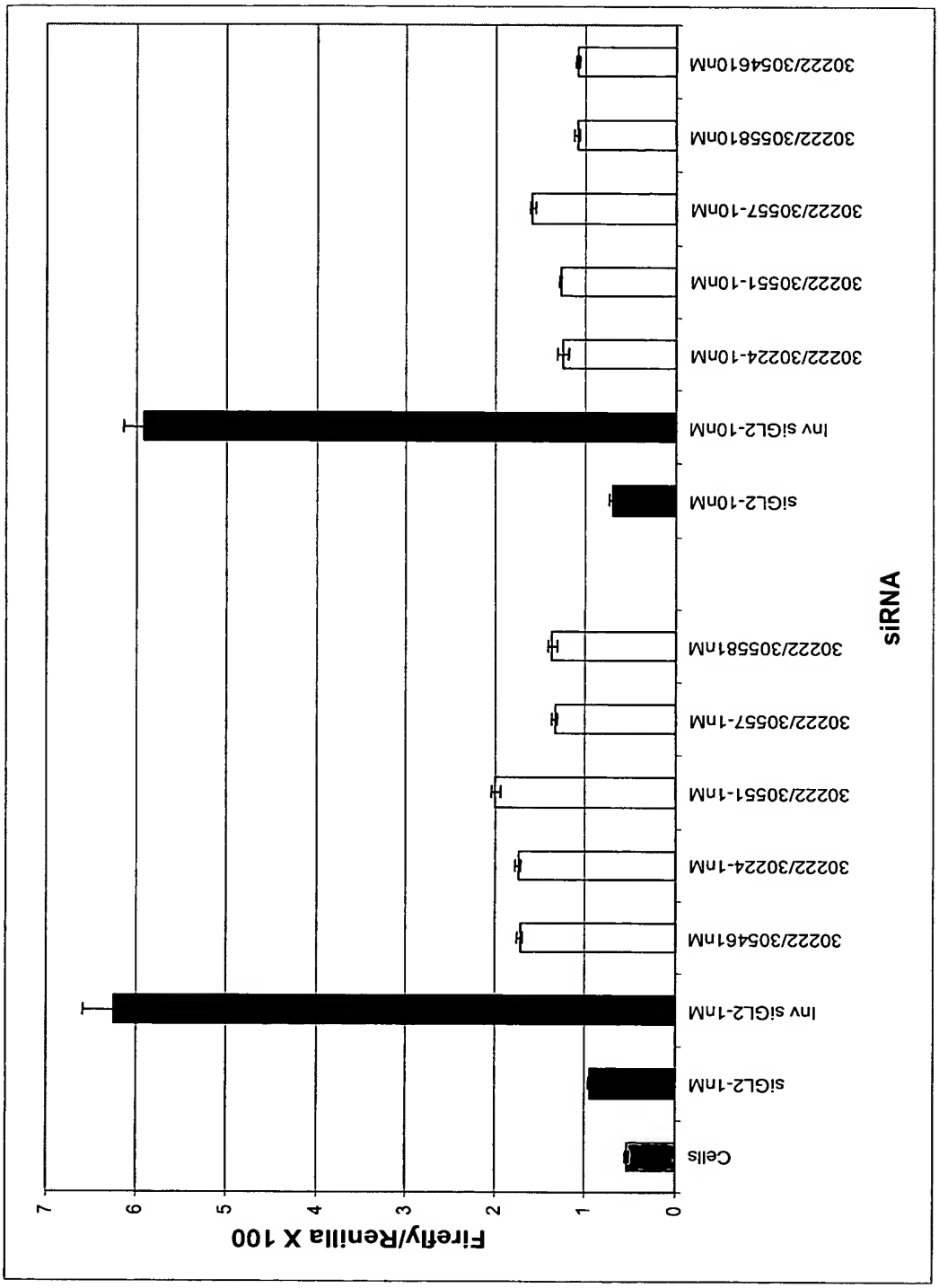

Firefly/Renilla X 100
0
1
2
3
4
5
6
7
siRNA
Cells
siGL2-1nM
Inv siGL2-1nM
30222/30546-1nM
30222/30224-1nM
30222/30551-1nM
30222/30557-1nM
30222/30558-1nM
siGL2-10nM
Inv siGL2-10nM
30222/30224-10nM
30222/30551-10nM
30222/30557-10nM
30222/30558-10nM
30222/30546-10nM

Figure 15

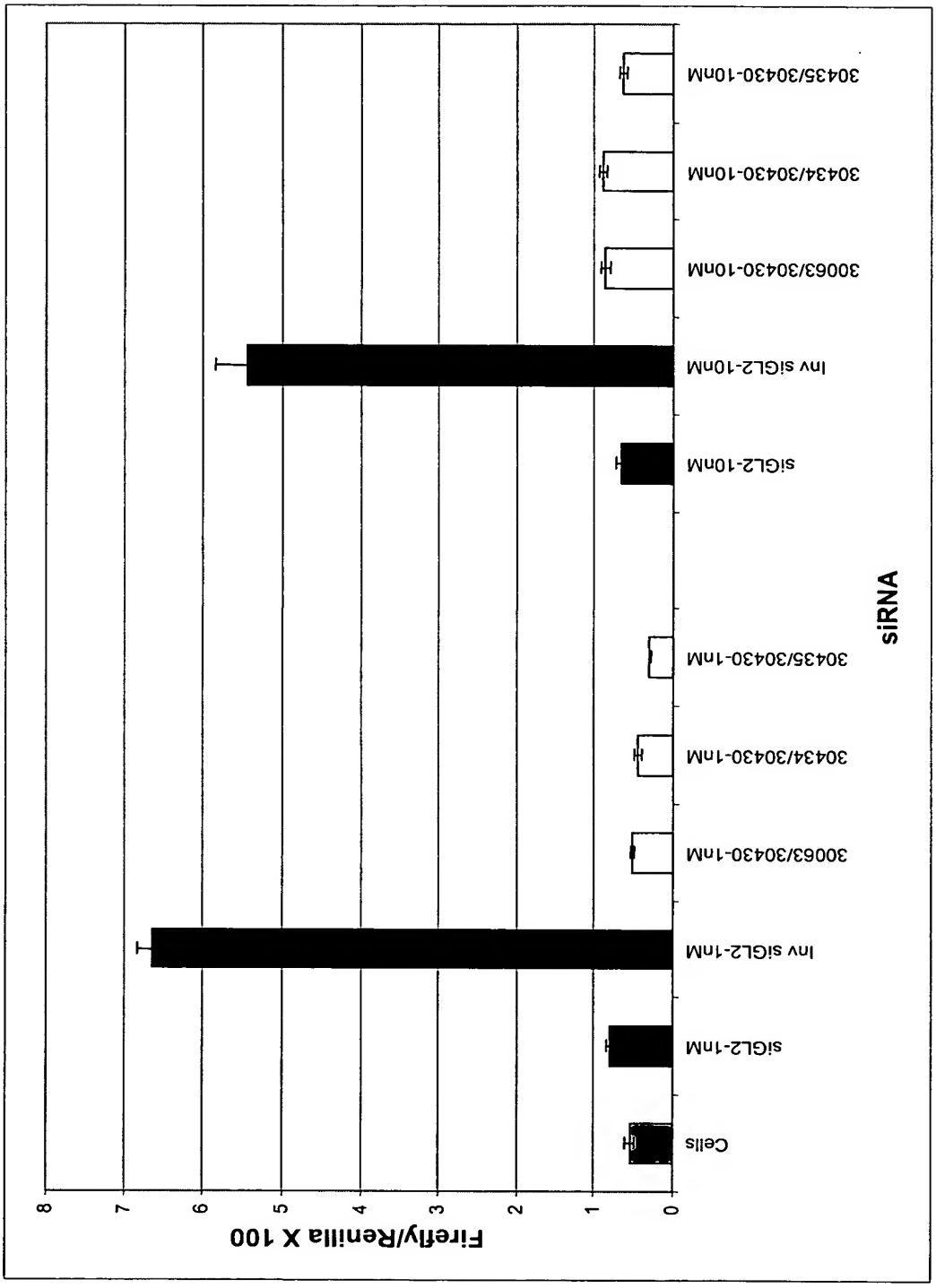

Firefly/Renilla X 100
0
1
2
3
4
5
6
7
8
siRNA
Cells
siGL2-1nM
Inv siGL2-1nM
30063/30430-1nM
30434/30430-1nM
30435/30430-1nM
siGL2-10nM
Inv siGL2-10nM
30063/30430-10nM
30434/30430-10nM
30435/30430-10nM

Figure 16

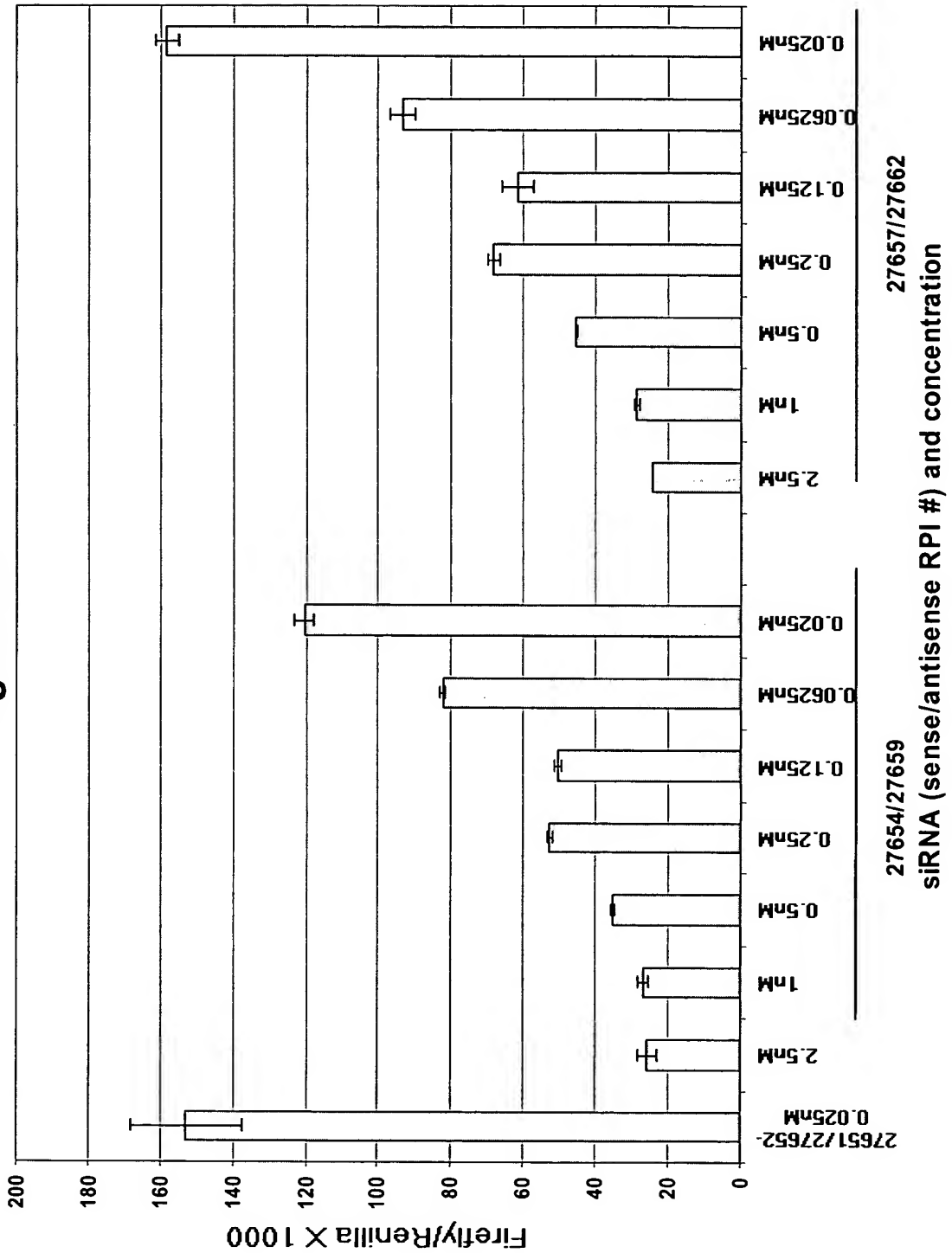

Firefly/Renilla X 1000
0
20
40
60
80
100
120
140
160
180
200
27651/27652-
0.025nM
2.5nM
1nM
0.5nM
0.25nM
0.125nM
0.0625nM
0.025nM
27654/27659
2.5nM
1nM
0.5nM
0.25nM
0.125nM
0.0625nM
0.025nM
27657/27662
siRNA (sense/antisense RPI #) and concentration

Figure 17

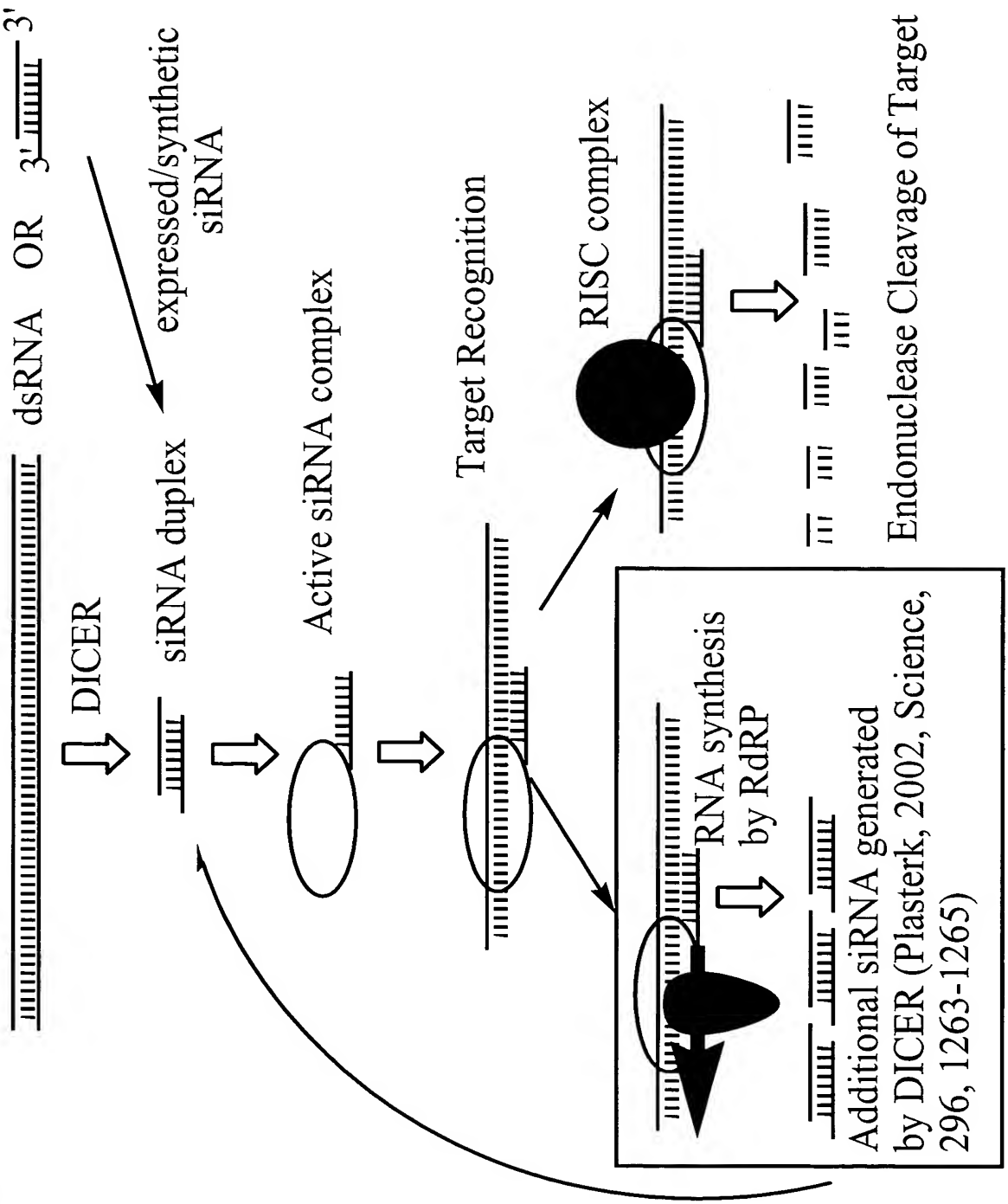

dsRNA OR 3' 3'
DICER
siRNA duplex
expressed/synthetic siRNA
Active siRNA complex
Target Recognition
RISC complex
RNA synthesis by RdRP
Endonuclease Cleavage of Target
Additional siRNA generated by DICER (Plasterk, 2002, Science, 296, 1263-1265)

Figure 18

A

SENSE STRAND (SEQ ID NO 471)
ALL PYRIMIDINES = 2'-O-ME OR 2'-FLUORO EXCEPT POSITIONS (N N)

5'- $N_s N_s N_s N_s N N N N N N N N N N N N N N N_s N_s N_s (N_s N)$ -3'

3'- L-(N_sN) N N N N N N N N N N N N N N N N $N_s N_s N_s N_s N$ -5'

ANTISENSE STRAND (SEQ ID NO 472)
ALL PYRIMIDINES = 2'-FLUORO EXCEPT POSITIONS (N N)

B

SENSE STRAND (SEQ ID NO 473)
ALL PYRIMIDINES = 2'-O-ME OR 2'-FLUORO EXCEPT POSITIONS (N N)

5'- N N N N N N N N N N N N N N N N N N N (N_sN) -3'

3'- L-(N N) N N N N N N N N N N N N N N N N N N N N N -5'

ANTISENSE STRAND (SEQ ID NO 474)
ALL PYRIMIDINES = 2'-FLUORO EXCEPT POSITIONS (N N)

C

SENSE STRAND (SEQ ID NO 475)
ALL PYRIMIDINES = 2'-O-ME OR 2'-FLUORO EXCEPT POSITIONS (N N)

5'- B-N N N N N N N N N N N N N N N N N N N (N N)-B -3'

3'- L-(N_sN) N N N N N N N N N N N N N N N N N N N N -5'

ANTISENSE STRAND (SEQ ID NO 476)
ALL PYRIMIDINES = 2'-FLUORO EXCEPT POSITIONS (N N)

D

SENSE STRAND (SEQ ID NO 477)
ALL PYRIMIDINES = 2'-FLUORO EXCEPT POSITIONS (N N) AND ALL PURINES = 2'-DEOXY

5'- B-N N N N N N N N N N N N N N N N N N N (N N)-B -3'

3'- L-(N_sN) N N N N N N N N N N N N N N N N N N N N -5'

ANTISENSE STRAND (SEQ ID NO 478)
ALL PYRIMIDINES = 2'-FLUORO AND ALL PURINES = 2'-O-ME EXCEPT POSITIONS (N N)

E

SENSE STRAND (SEQ ID NO 479)
ALL PYRIMIDINES = 2'-FLUORO EXCEPT POSITIONS (N N)

5'- B-N N N N N N N N N N N N N N N N N N N (N N)-B -3'

3'- L-(N_sN) N N N N N N N N N N N N N N N N N N N N -5'

ANTISENSE STRAND (SEQ ID NO 480)
ALL PYRIMIDINES = 2'-FLUORO AND ALL PURINES = 2'-O-ME EXCEPT POSITIONS (N N)

F

SENSE STRAND (SEQ ID NO 477)
ALL PYRIMIDINES = 2'-FLUORO EXCEPT POSITIONS (N N) AND ALL PURINES = 2'-DEOXY

5'- B-N N N N N N N N N N N N N N N N N N N (N N)-B -3'

3'- L-(N_sN) N N N N N N N N N N N N N N N N N N N N -5'

ANTISENSE STRAND (SEQ ID NO 481)
ALL PYRIMIDINES = 2'-FLUORO EXCEPT POSITIONS (N N) AND ALL PURINES = 2'-DEOXY

POSITIONS (NN) CAN COMPRISE ANY NUCLEOTIDE, SUCH AS DEOXYNUCLEOTIDES (eg. THYMIDINE) OR UNIVERSAL BASES
B = ABASIC, INVERTED ABASIC, INVERTED NUCLEOTIDE OR OTHER TERMINAL CAP THAT IS OPTIONALLY PRESENT
L = GLYCERYL or B THAT IS OPTIONALLY PRESENT
S = PHOSPHOROTHIOATE OR PHOSPHORODITHIOATE that is optionally absent

Figure 19

A

SENSE STRAND (SEQ ID NO 482)

5'- $c_S c_S c_S c_S$G G G A G G u c u c G u $A_S G_S A_S T_S T$ -3'

3'- L-$T_S T$ G G G G *c c c u c c* A G A G $c_S A_S u_S c_S u$ -5'

ANTISENSE STRAND (SEQ ID NO 483)

B

SENSE STRAND (SEQ ID NO 484)

5'- c c c c G G G A G G u c u c G u A G A $T_S T$ -3'

3'- L-*T T* G G G G *c c c u c c* A G A G *c* A *u c u* -5'

ANTISENSE STRAND (SEQ ID NO 485)

C

SENSE STRAND (SEQ ID NO 486)

5'- iB-c c c c G G G A G G u c u c G u A G A *T T*-iB -3'

3'- L-$T_S T$ G G G G *c c c u c c* A G A G *c* A *u c u* -5'

ANTISENSE STRAND (SEQ ID NO 487)

D

SENSE STRAND (SEQ ID NO 488)

5'- iB-*c c c c G G G A G G u c u c G u A G A T T*-iB -3'

3'- L-$T_S T$ g g g g *c c c u c c* a g a g *c* a *u c u* -5'

ANTISENSE STRAND (SEQ ID NO 489)

E

SENSE STRAND (SEQ ID NO 490)

5'- iB-*c c c c* G G G A G G *u c u c* G *u* A G A *T T*-iB -3'

3'- L-$T_S T$ g g g g *c c c u c c* a g a g *c* a *u c u* -5'

ANTISENSE STRAND (SEQ ID NO 491)

F

SENSE STRAND (SEQ ID NO 488)

5'- iB-*c c c c G G G A G G u c u c G u A G A T T*-iB -3'

3'- L-$T_S T$ *G G G G c c c u c c A G A G c A u c u* -5'

ANTISENSE STRAND (SEQ ID NO 492)

lower case = 2'-O-Methyl or 2'-deoxy-2'-fluoro

italic lower case = 2'-deoxy-2'-fluoro

underline = 2'-O-methyl

ITALIC UPPER CASE = DEOXY
iB = INVERTED DEOXYABASIC
L = GLYCERYL MOIETY or iB OPTIONALLY PRESENT
S = PHOSPHOROTHIOATE OR
PHOSPHORODITHIOATE OPTIONALLY PRESENT

Figure 20

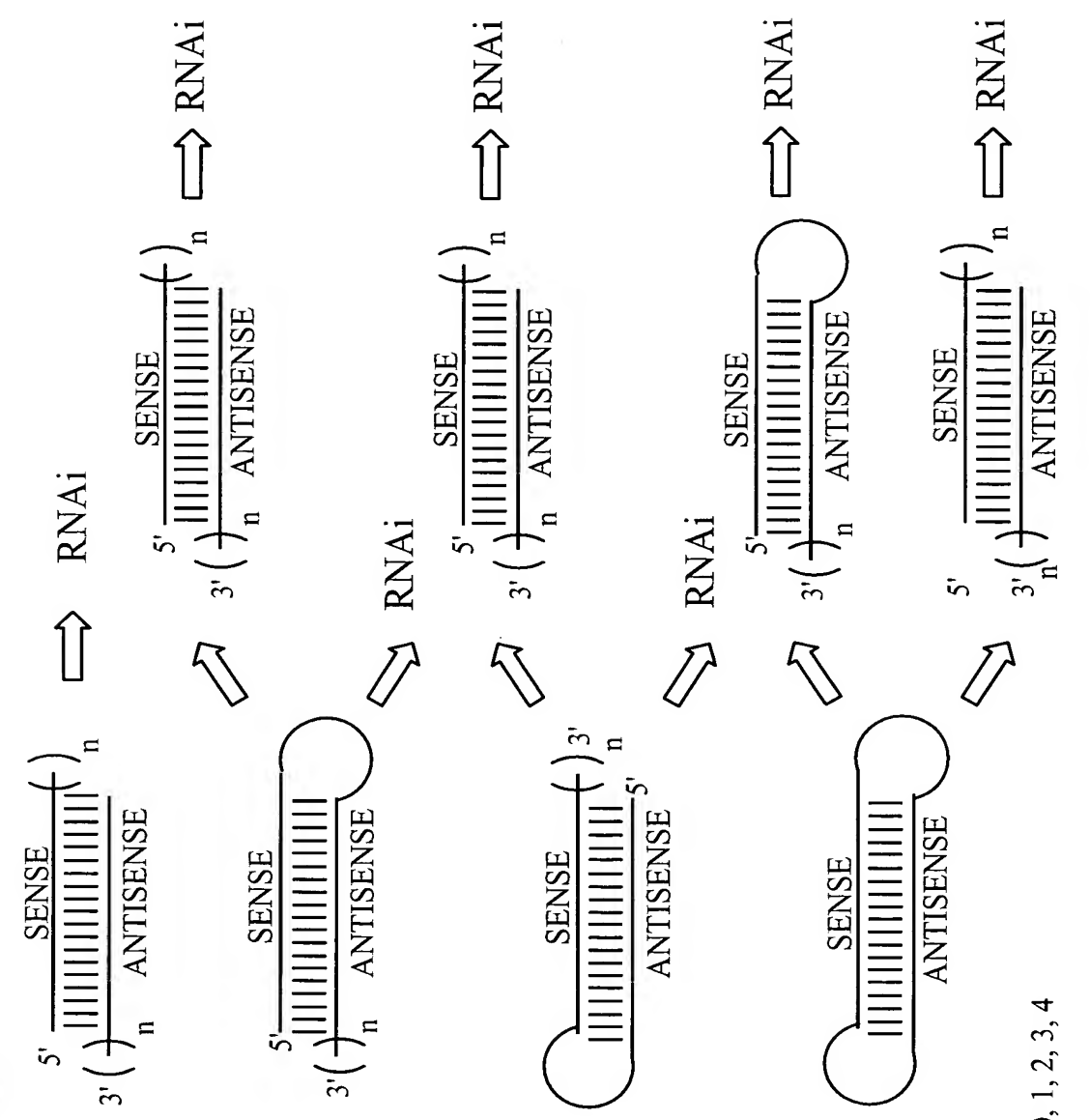


Figure 21: Target site Selection using siRNA

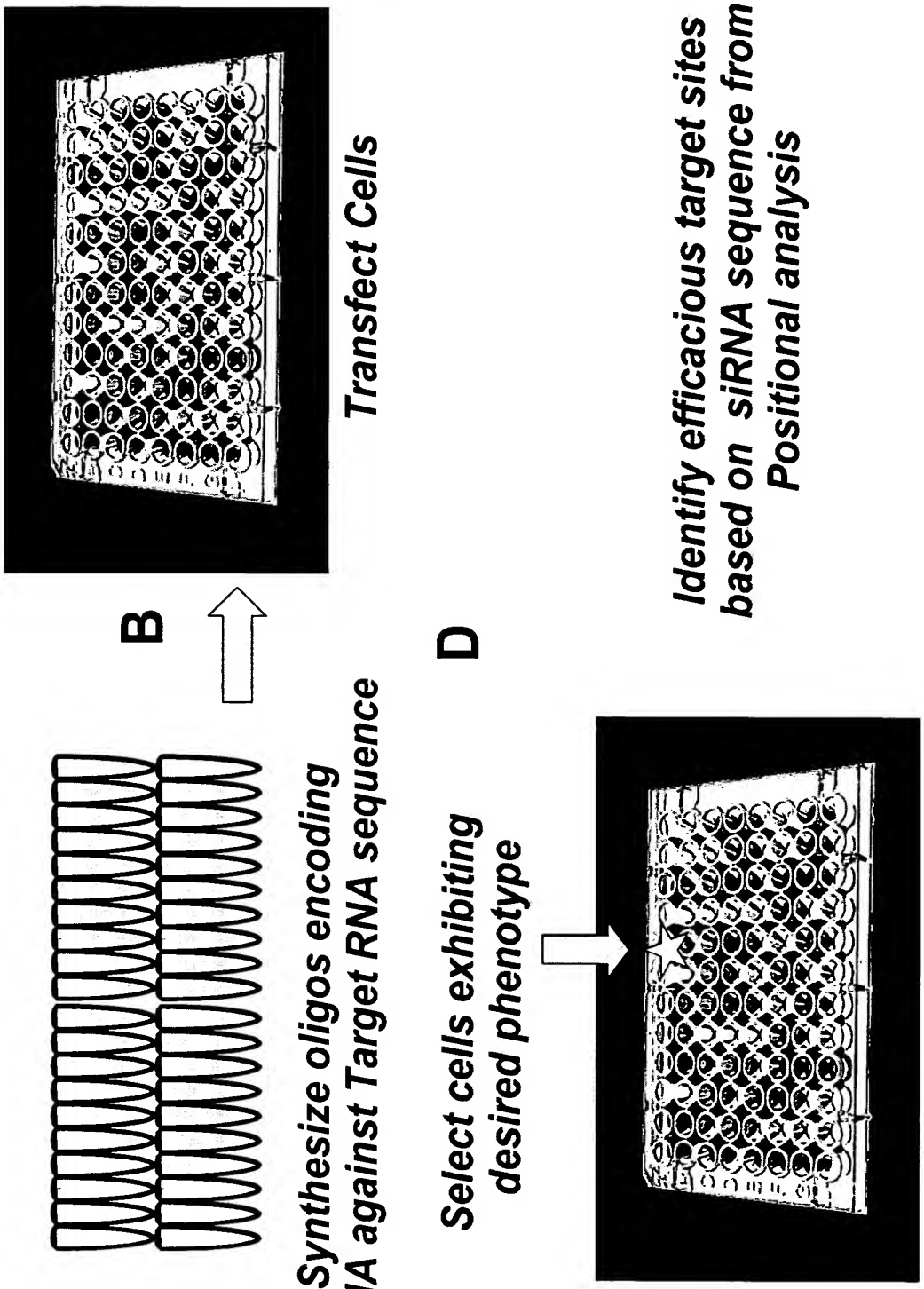
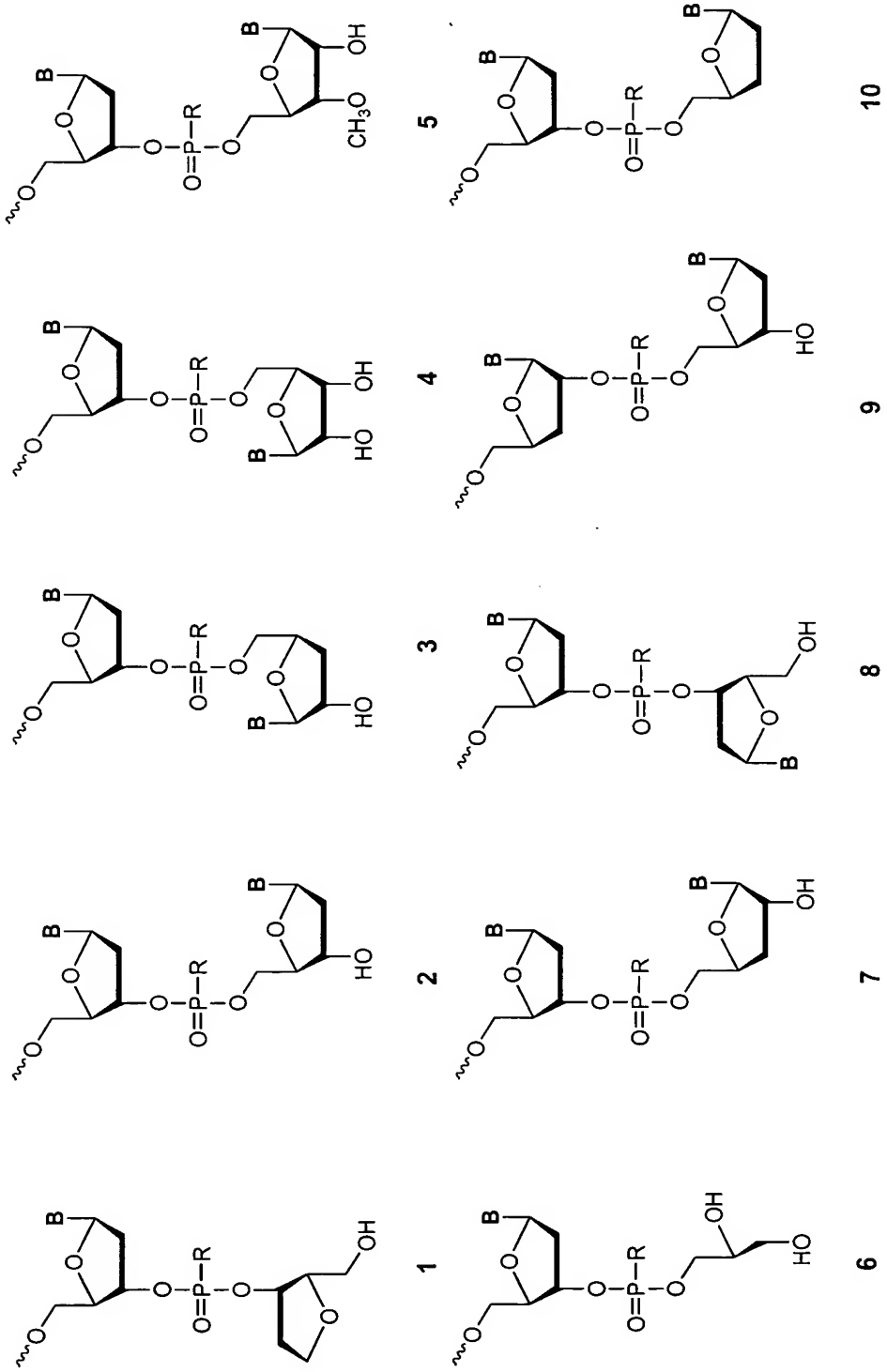


Figure 22



R = O, S, N, alkyl, substituted alkyl, O-alkyl, S-alkyl, alkaryl, or aralkyl
B = Independently any nucleotide base, either naturally occurring or chemically modified, or optionally H (abasic).

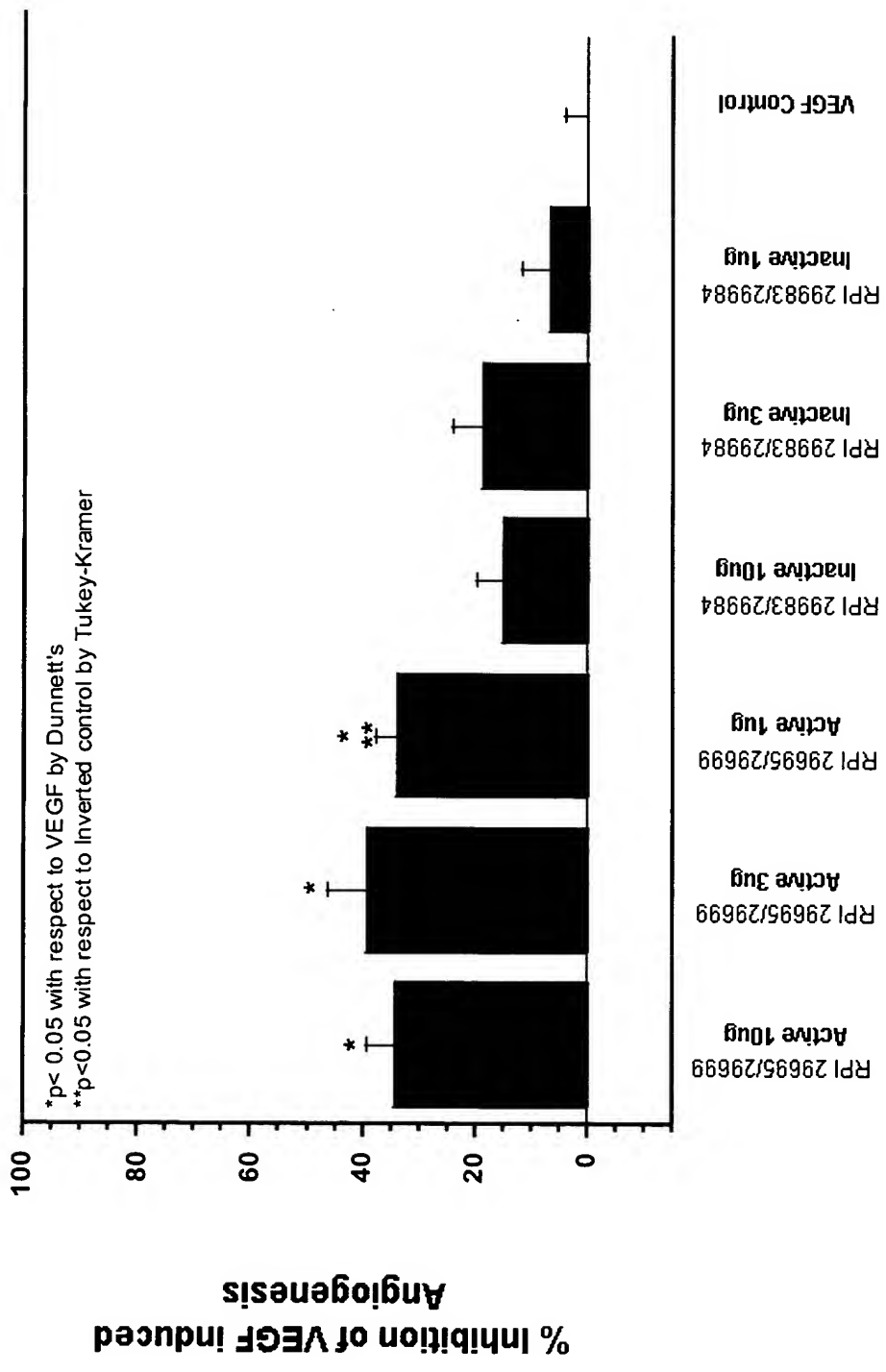


Figure 23: Inhibition of VEGF-Induced Angiogenesis by siRNAs

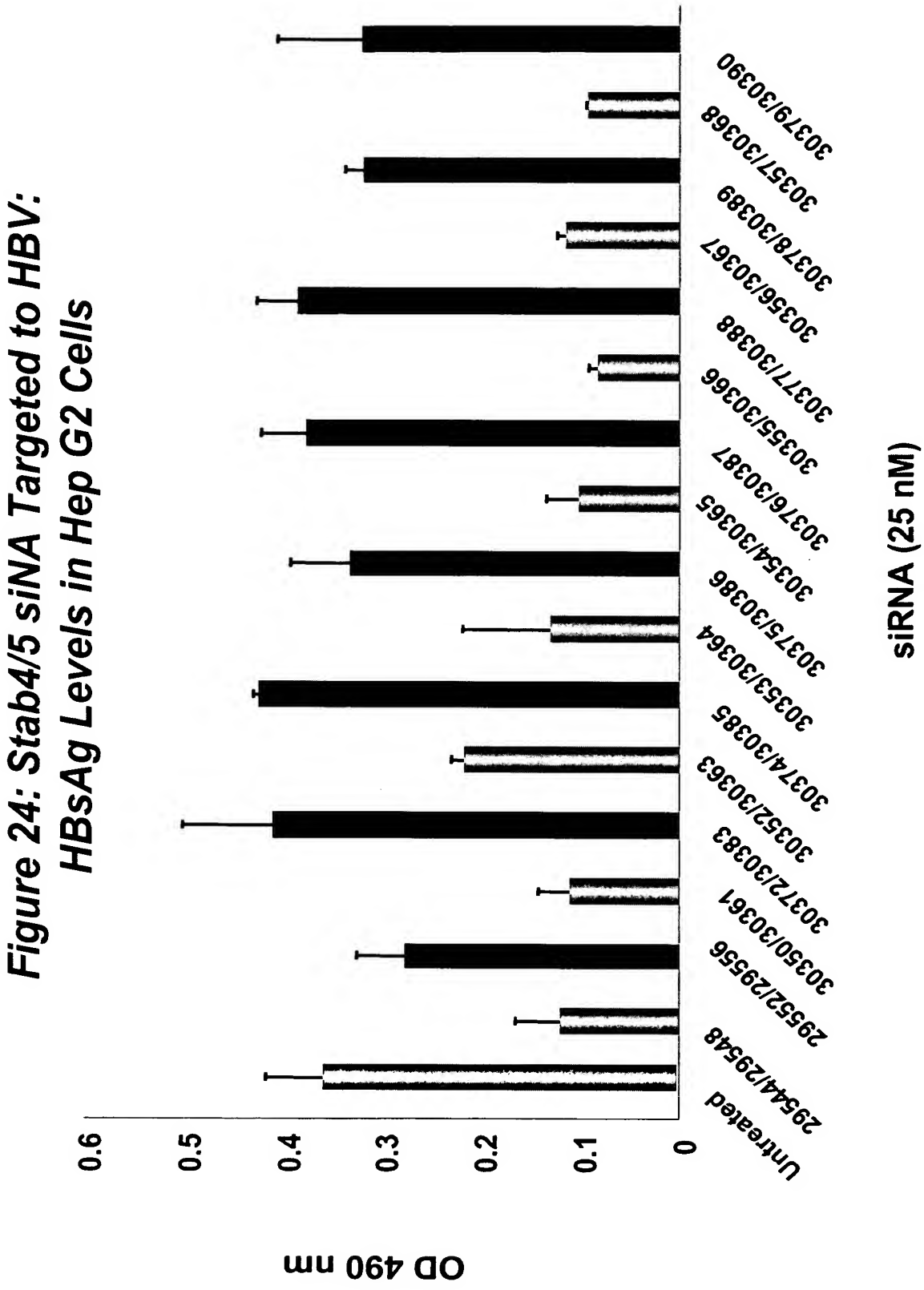


Figure 24: Stab4/5 siNA Targeted to HBV: HBsAg Levels in Hep G2 Cells
OD 490 nm
0.6
0.5
0.4
0.3
0.2
0.1
0
siRNA (25 nM)
Untreated
29544/29548
29552/29556
30350/30361
30372/30383
30352/30363
30374/30385
30353/30364
30375/30386
30354/30365
30376/30387
30355/30366
30377/30388
30356/30367
30378/30389
30357/30368
30379/30390

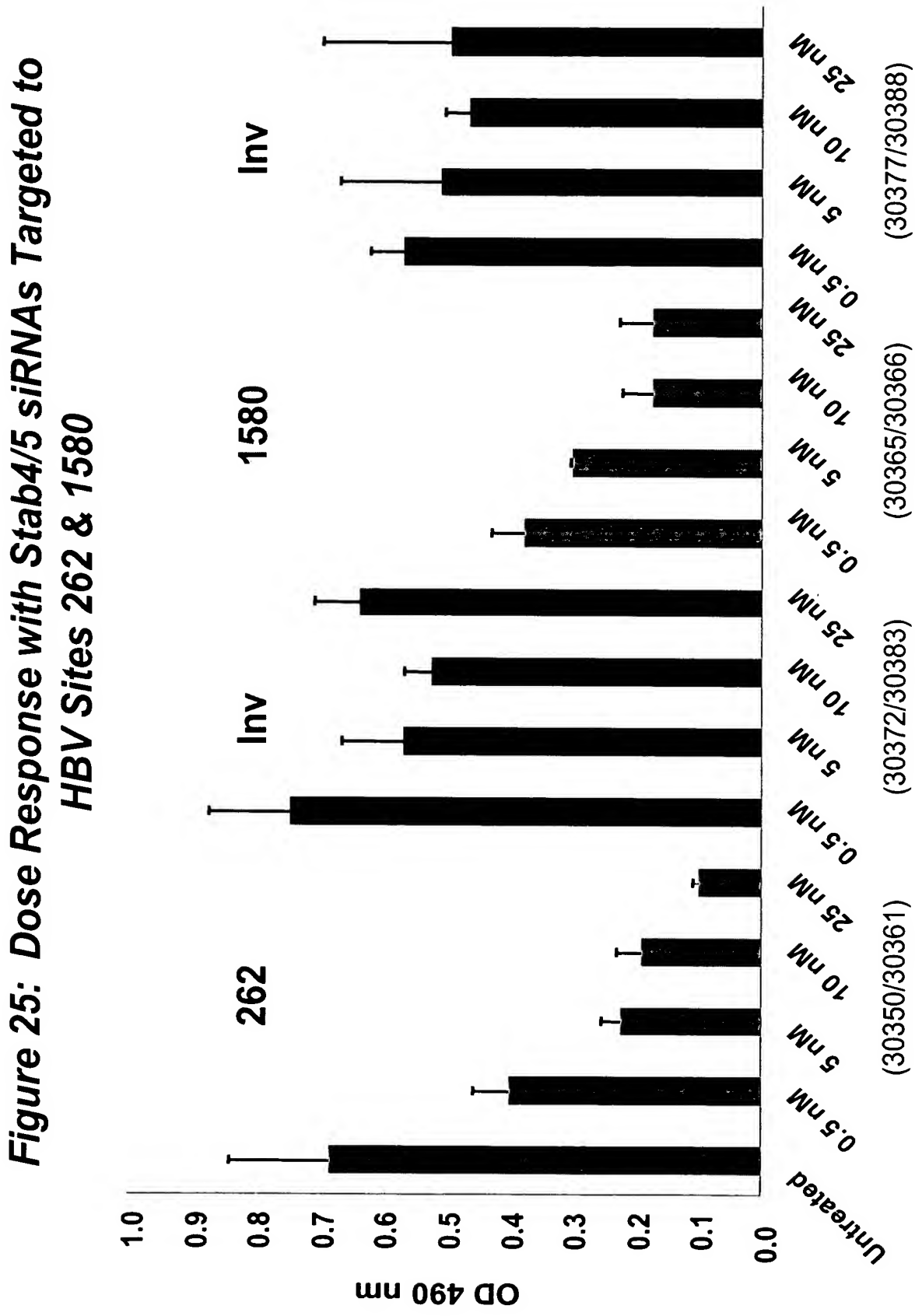


Figure 25: Dose Response with Stab4/5 siRNAs Targeted to HBV Sites 262 & 1580
OD 490 nm
1.0
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0.0
262
Inv
1580
Inv
Untreated
0.5 nM
5 nM
10 nM
25 nM
(30350/30361)
(30372/30383)
(30365/30366)
(30377/30388)

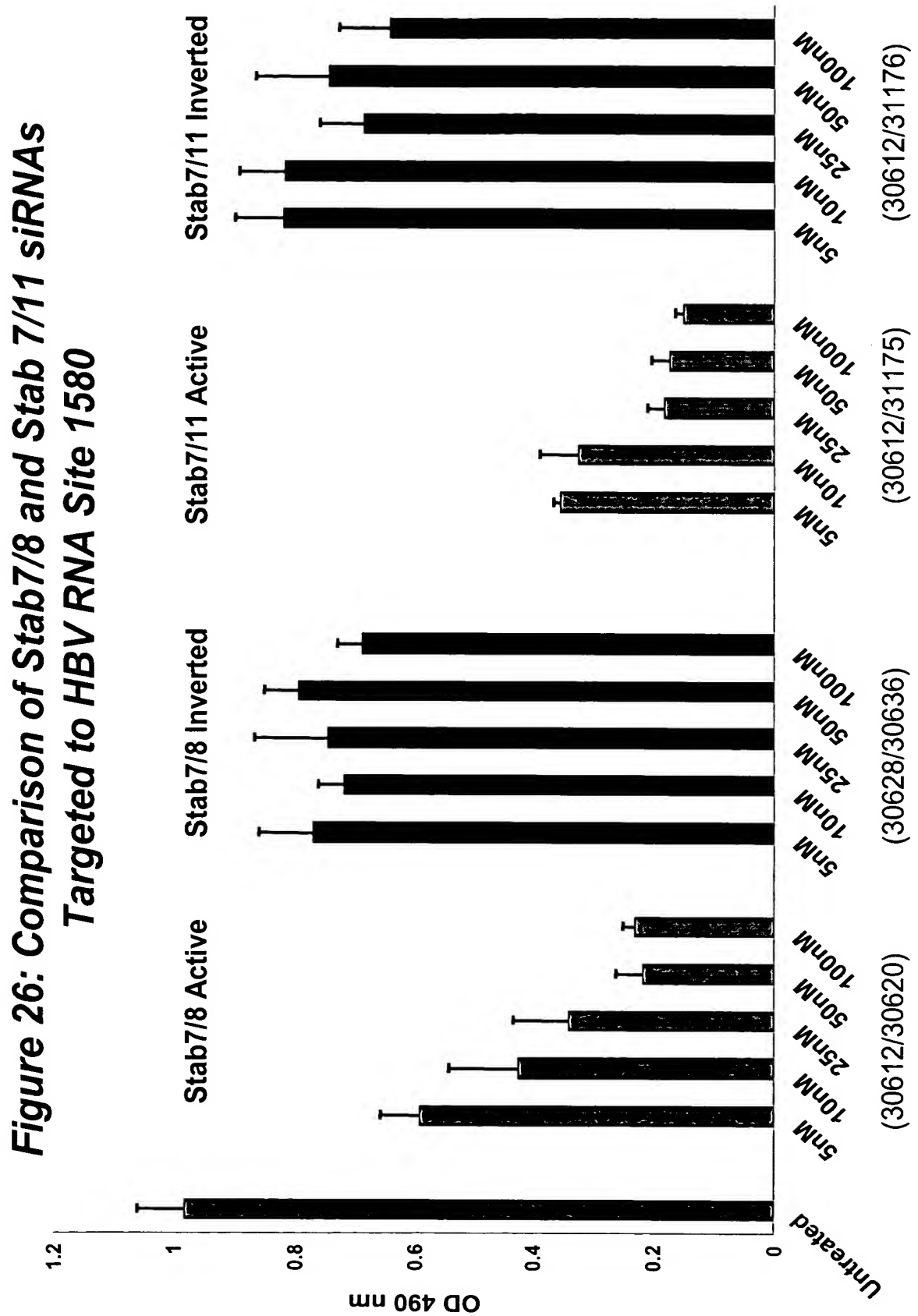


Figure 26: Comparison of Stab7/8 and Stab 7/11 siRNAs Targeted to HBV RNA Site 1580
OD 490 nm
1.2
1
0.8
0.6
0.4
0.2
0
Untreated
5nM
10nM
25nM
50nM
100nM
Stab7/8 Active
Stab7/8 Inverted
Stab7/11 Active
Stab7/11 Inverted
(30612/30620)
(30628/30636)
(30612/31175)
(30612/31176)

Figure 27: Modification Strategy

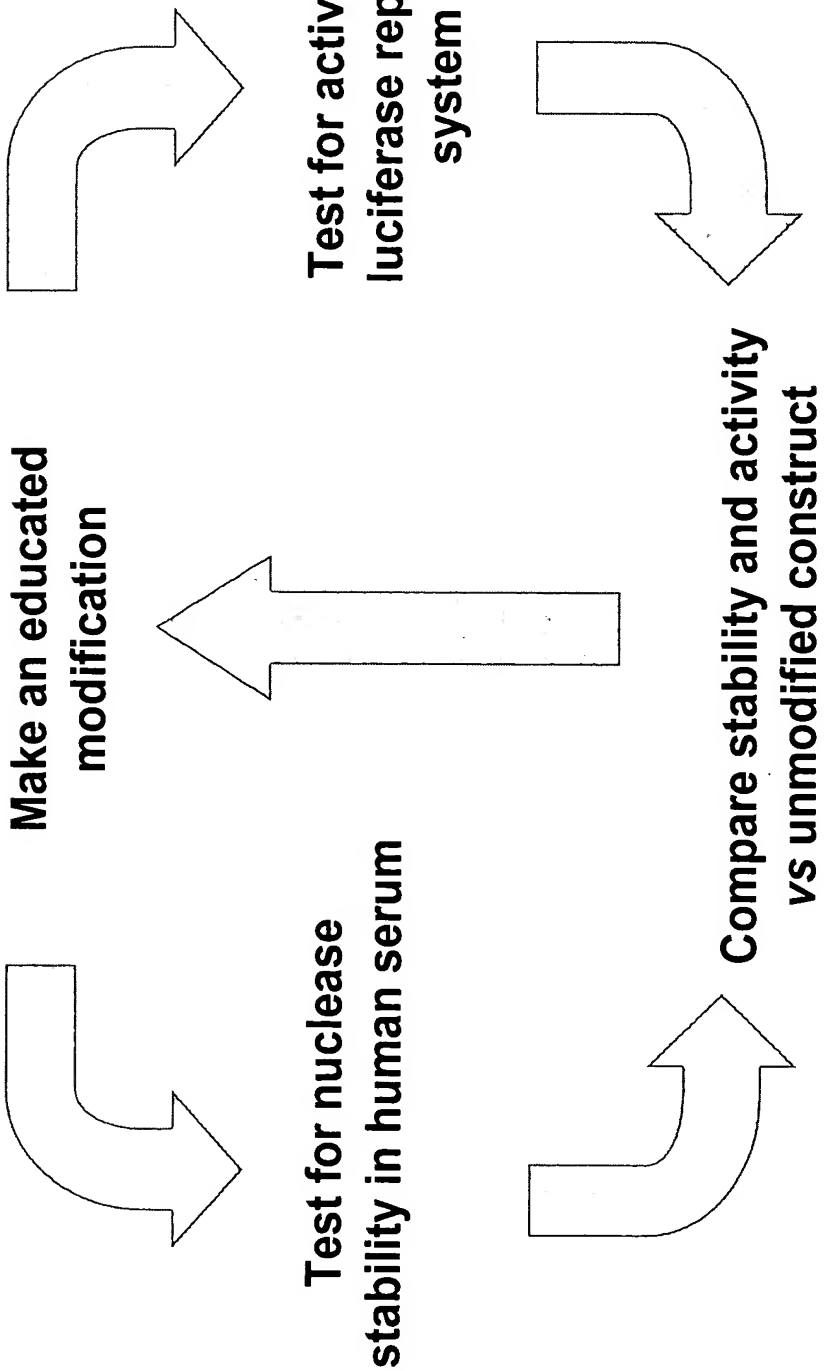


Figure 28: Duration of siRNA Effect
All-Ribo vs. Stab4/5 HBV Site 1580: HBsAg Levels

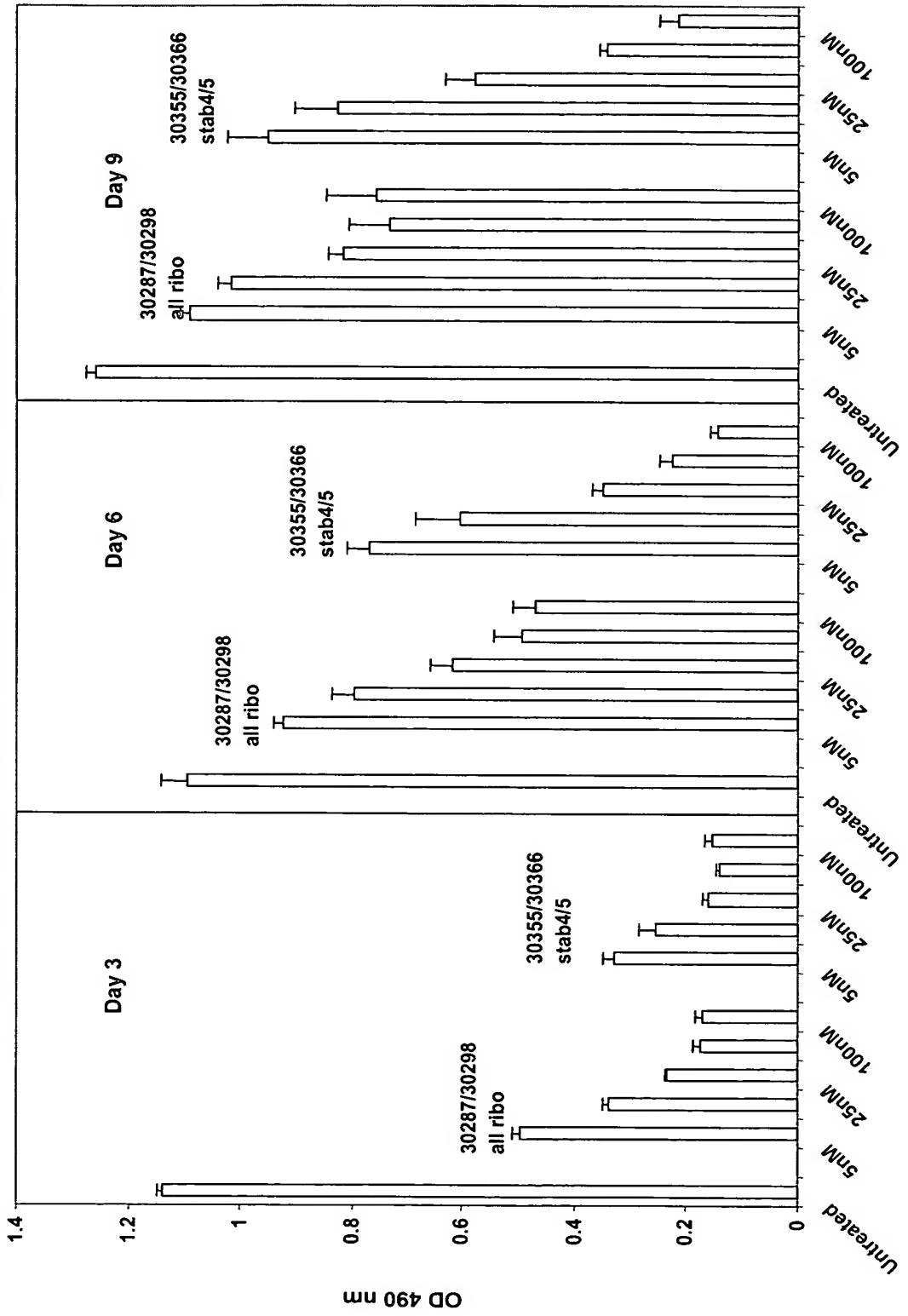
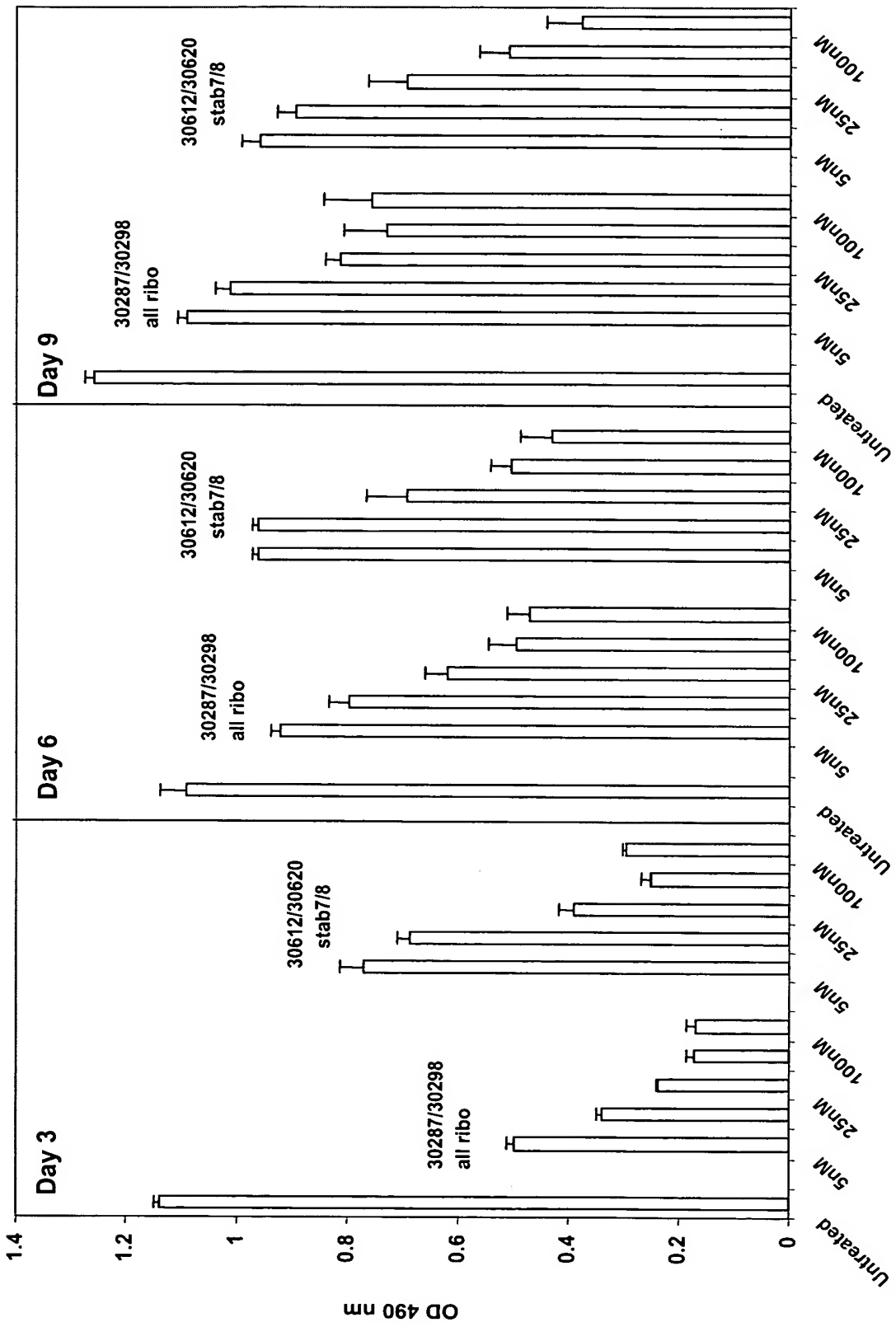


Figure 29: Duration of siRNA Effect
All-Ribo vs. Stab7/8 HBV Site 1580: HBsAg Levels



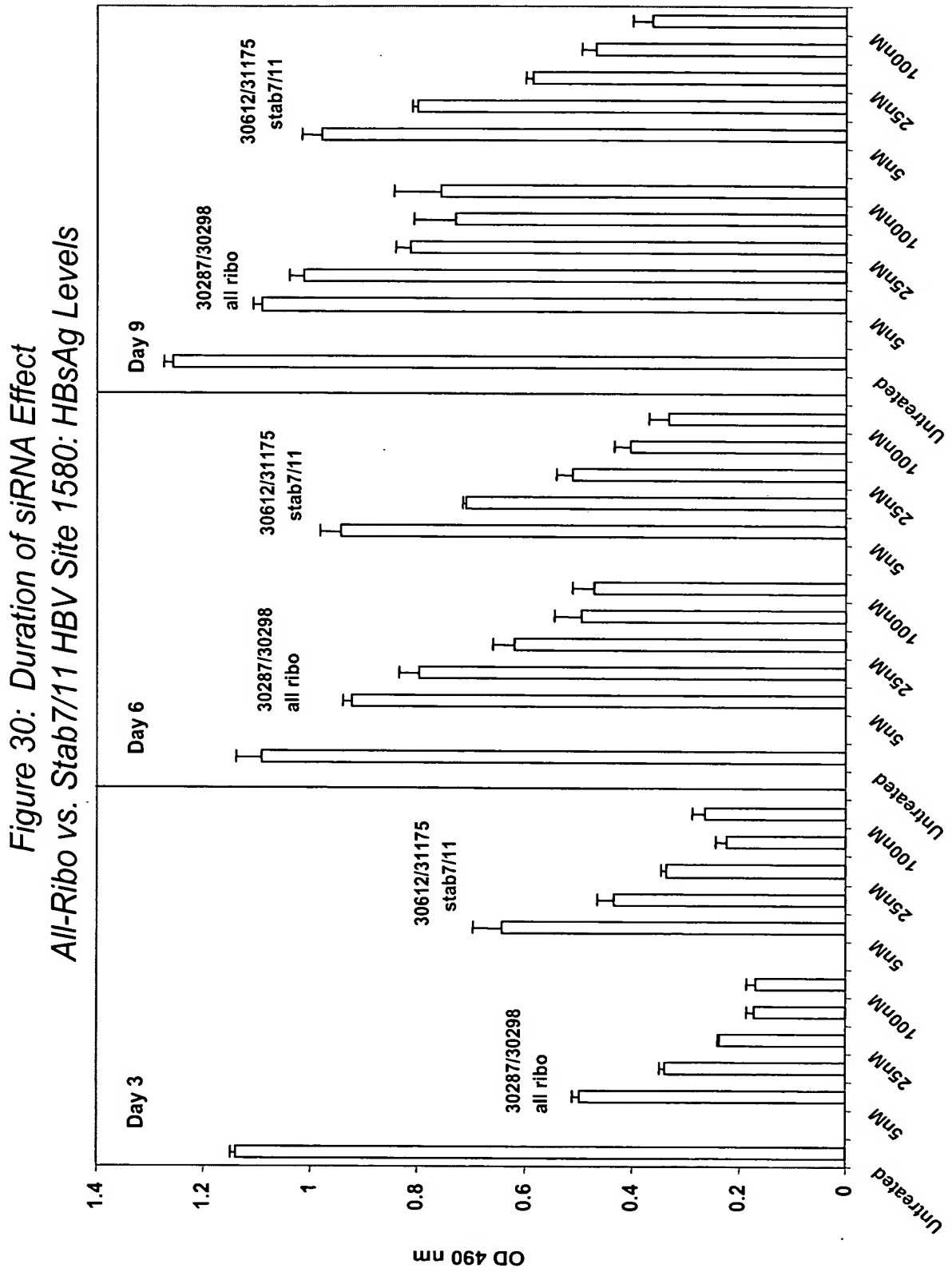


Figure 30: Duration of siRNA Effect
All-Ribo vs. Stab7/11 HBV Site 1580: HBsAg Levels
OD 490 nm
1.4
1.2
1
0.8
0.6
0.4
0.2
0
Day 3
Day 6
Day 9
30287/30298 all ribo
30612/31175 stab7/11
Untreated
5nM
25nM
100nM

Figure 31: Duration of siRNA Effect
All-Ribo vs. Stab9/10 HBV Site 1580: HBsAg Levels

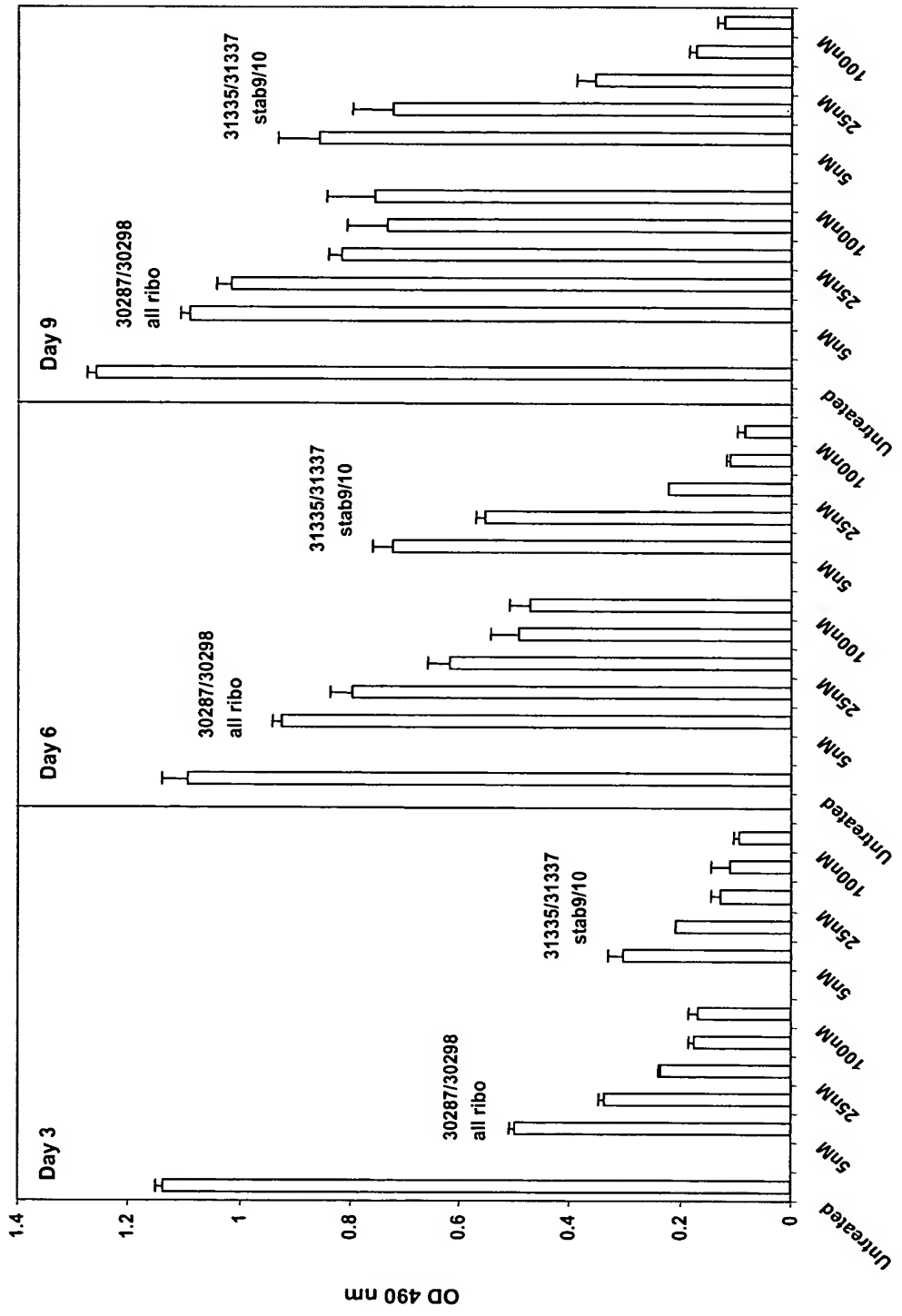


Figure 32 : siRNAs targeting HCV chimera

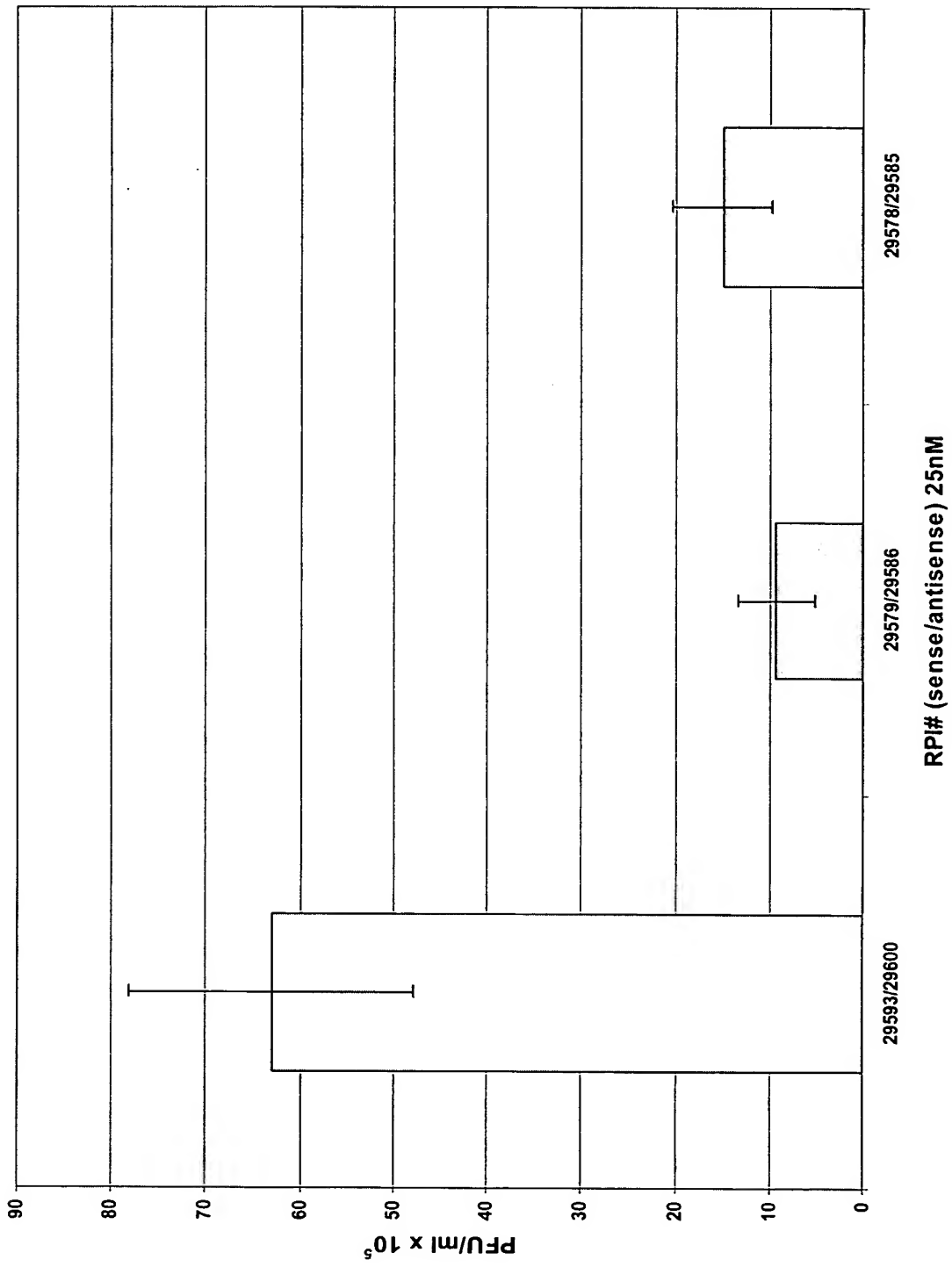


Figure 33: HCV siRNA dose response

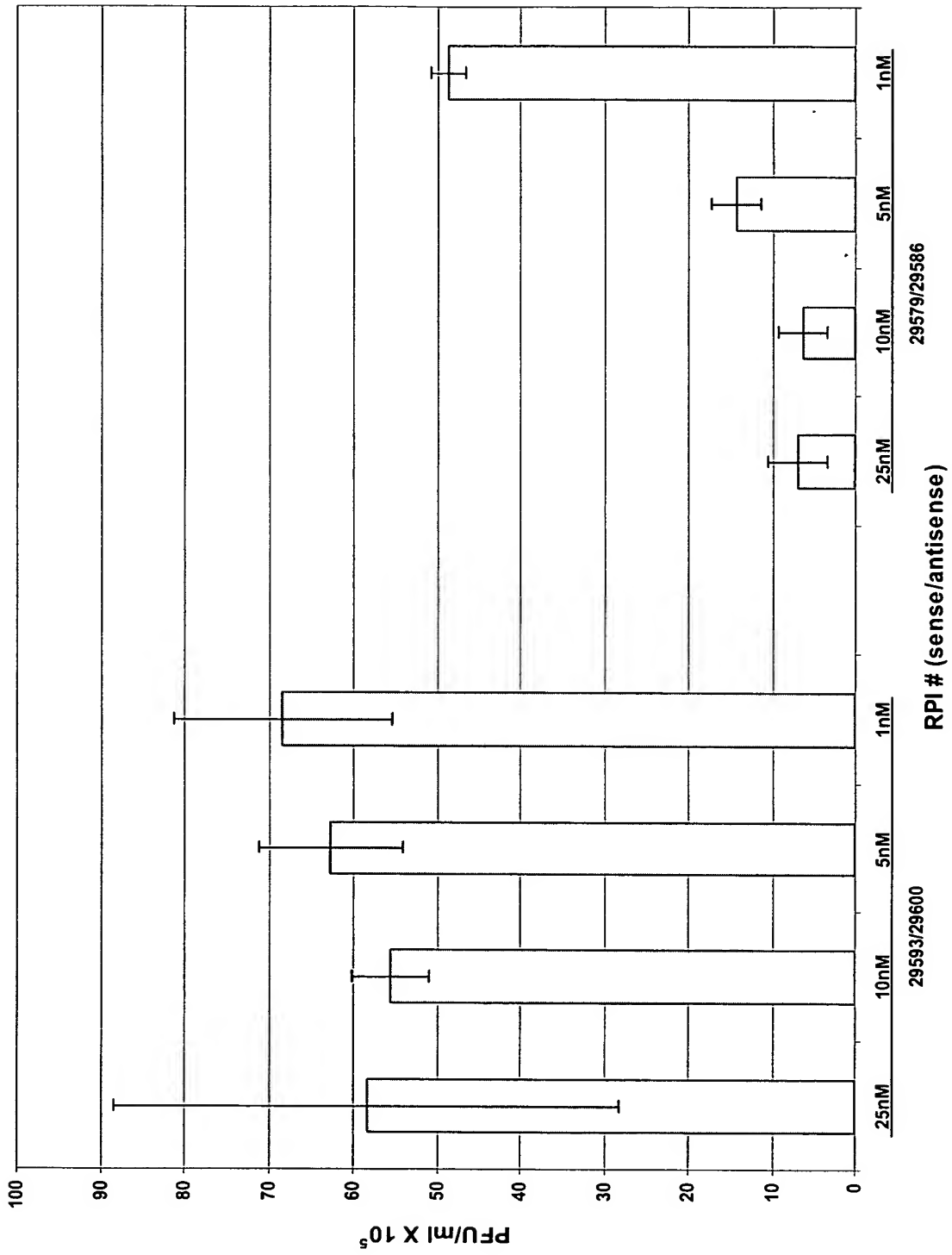


Figure 34: Chemically Modified siRNA targeting HCV chimera

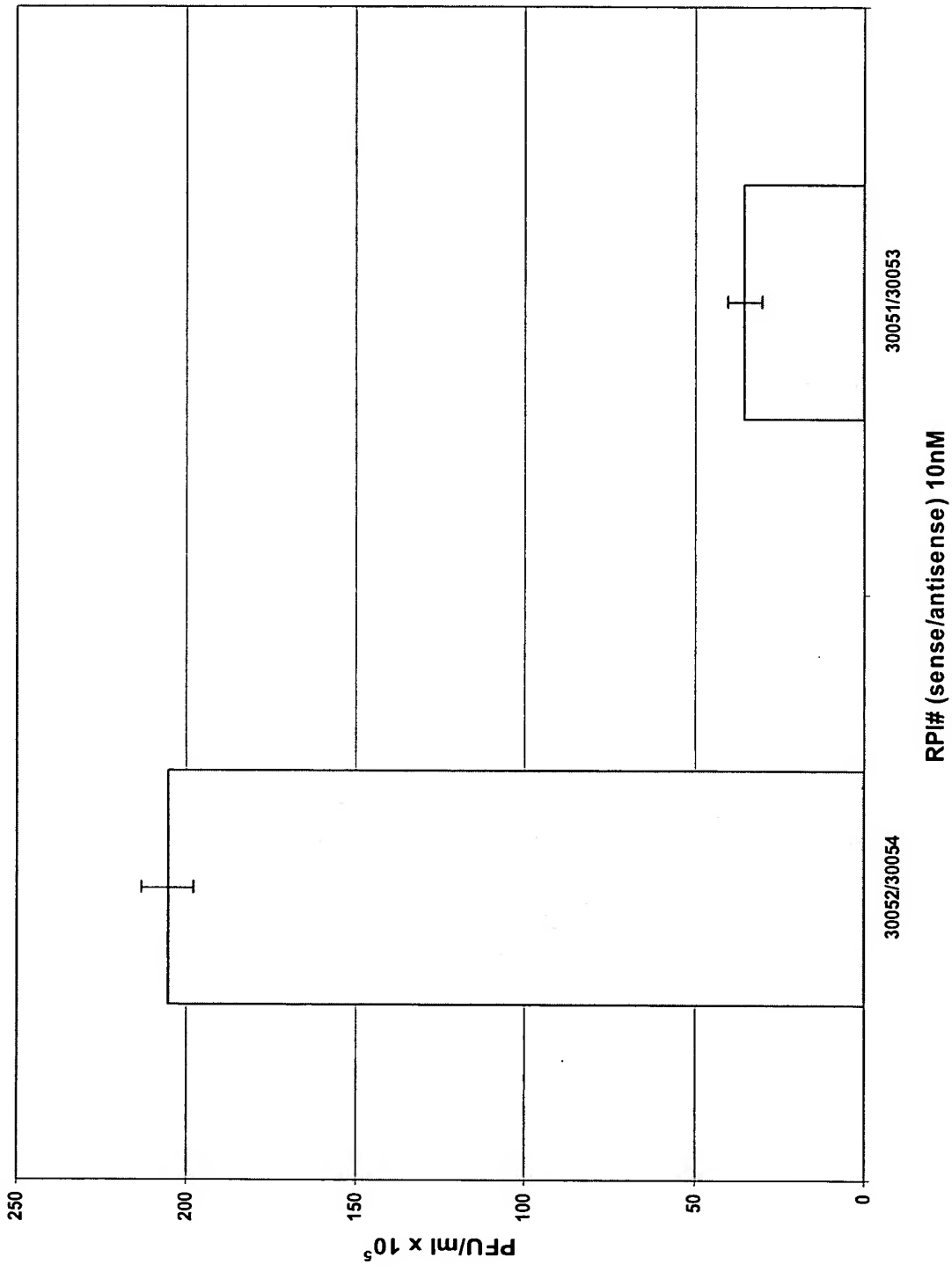


Figure 35: Chemically Modified siRNA targeting HCV chimera

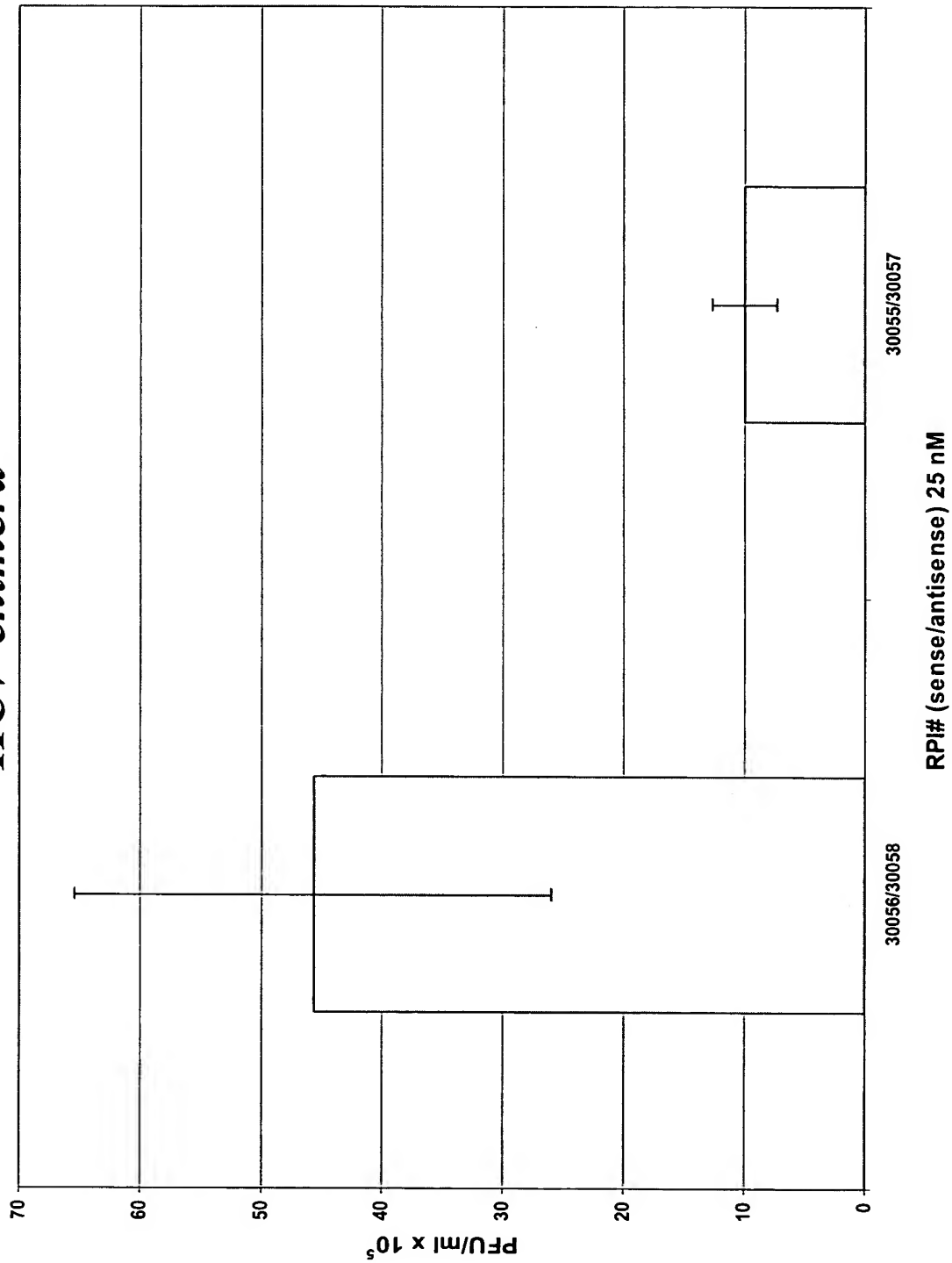


Figure 36: Chemically Modified siRNA targeting HCV chimera

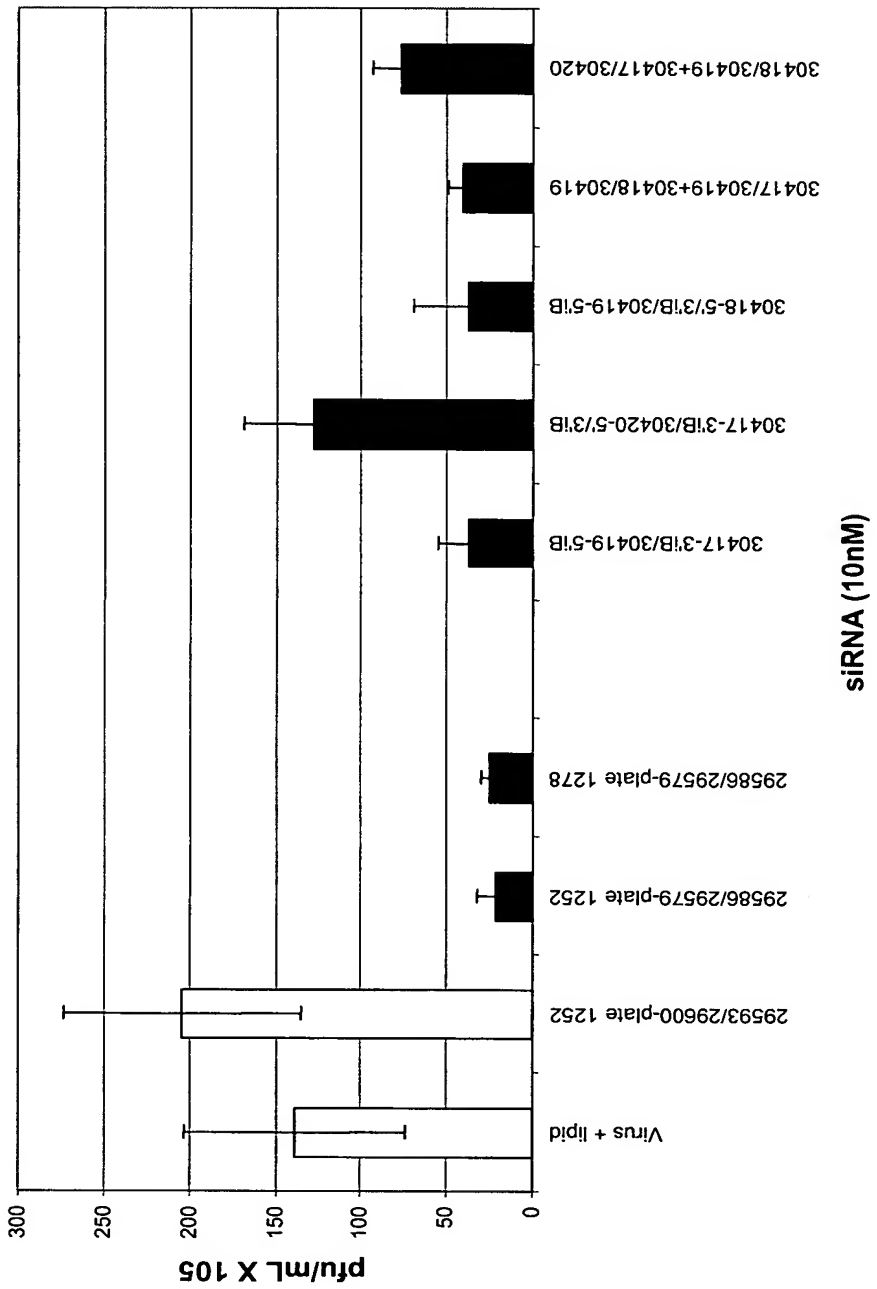
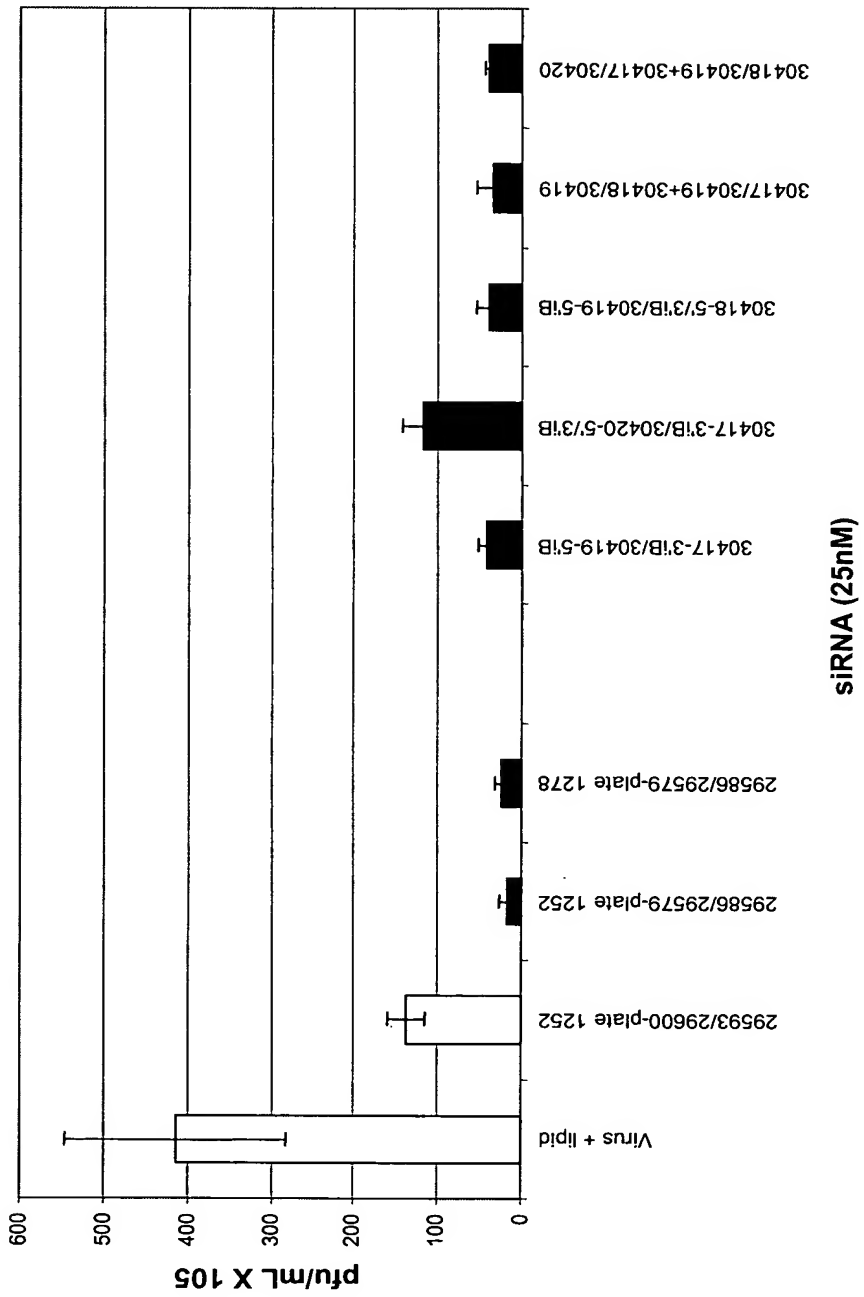


Figure 37: Chemically Modified siRNA targeting HCV chimera



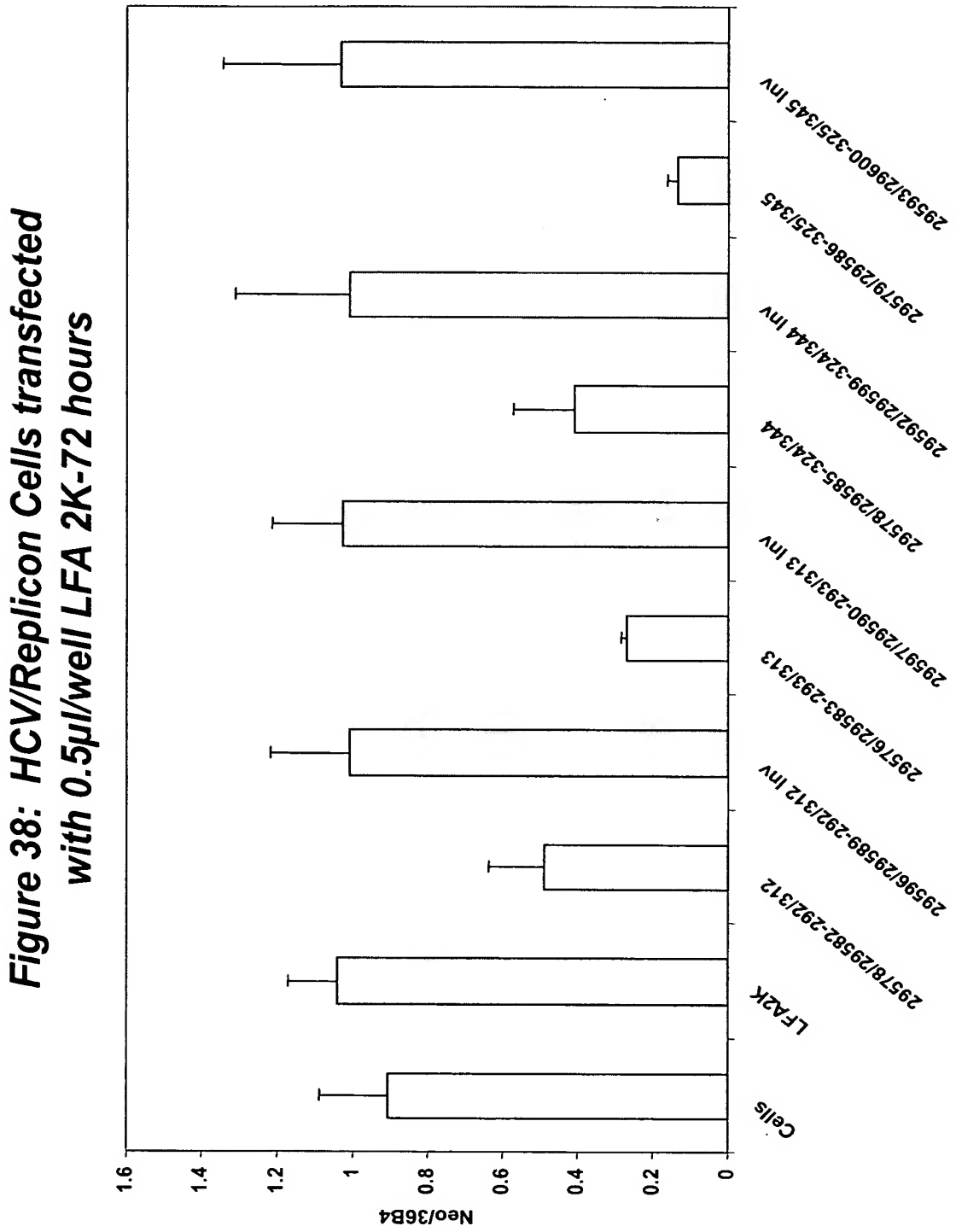


Figure 38: HCV/Replicon Cells transfected with 0.5µl/well LFA 2K-72 hours

Figure 39: Dose Response with Stab4/5 siNA Leads in HCV Subgenomic Replicon

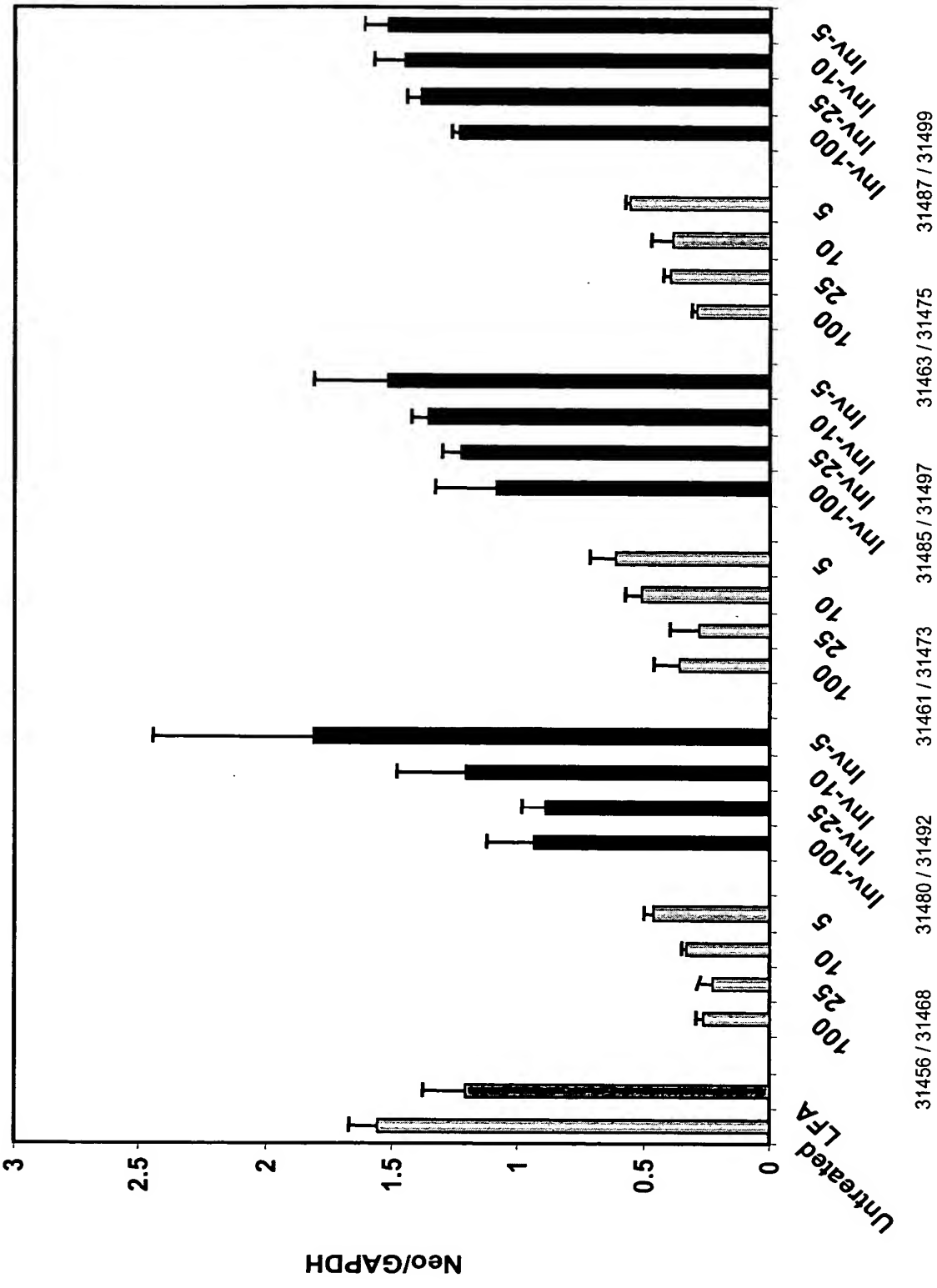


Figure 40: Activity of Stab 7/8 siNA Leads in HCV Subgenomic Replicon

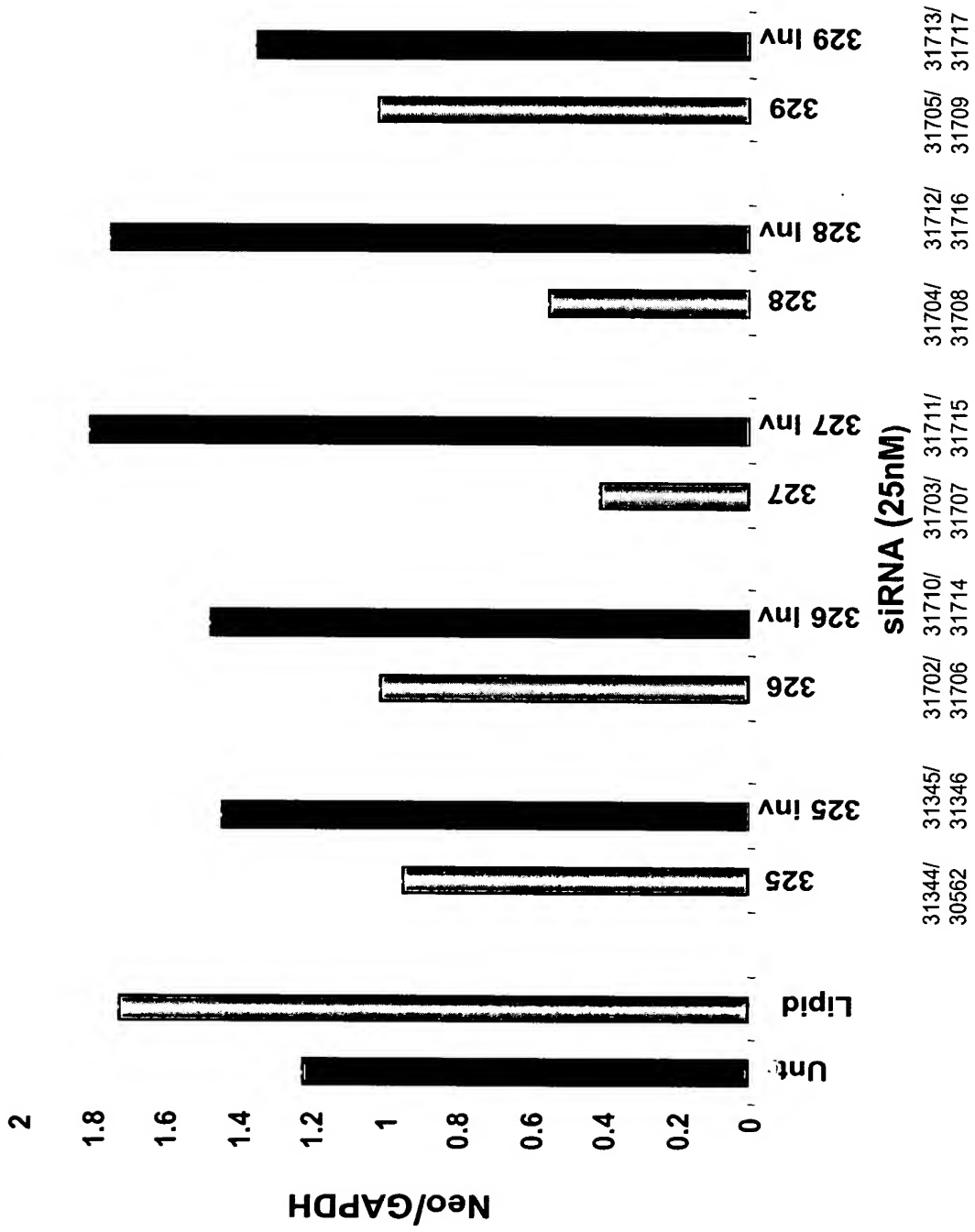


Figure 41: Dose Response with Fully Modified HCV Site 327 siNA

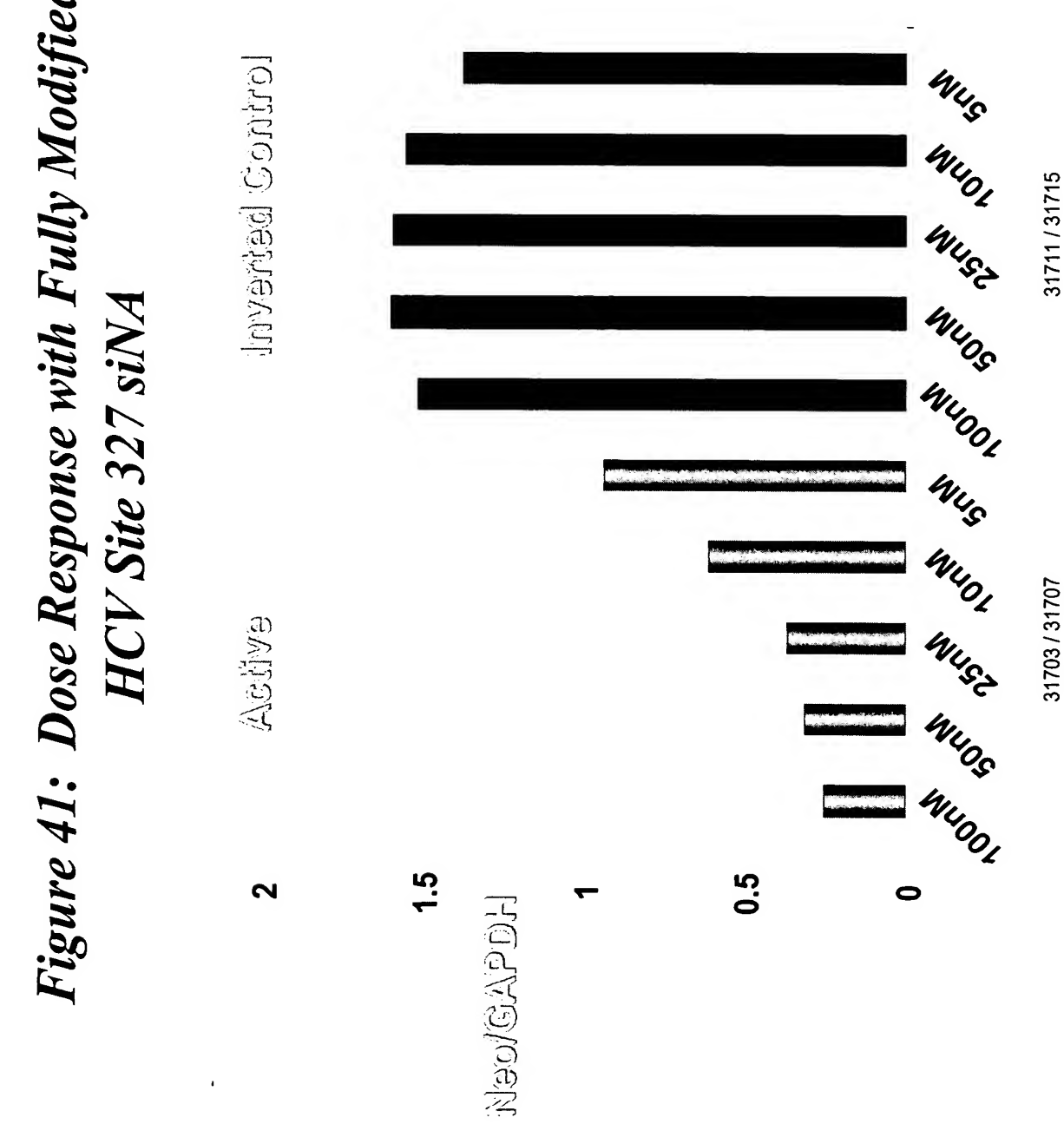


Figure 42: Solid Phase Post-synthetic conjugation of pteroic acid

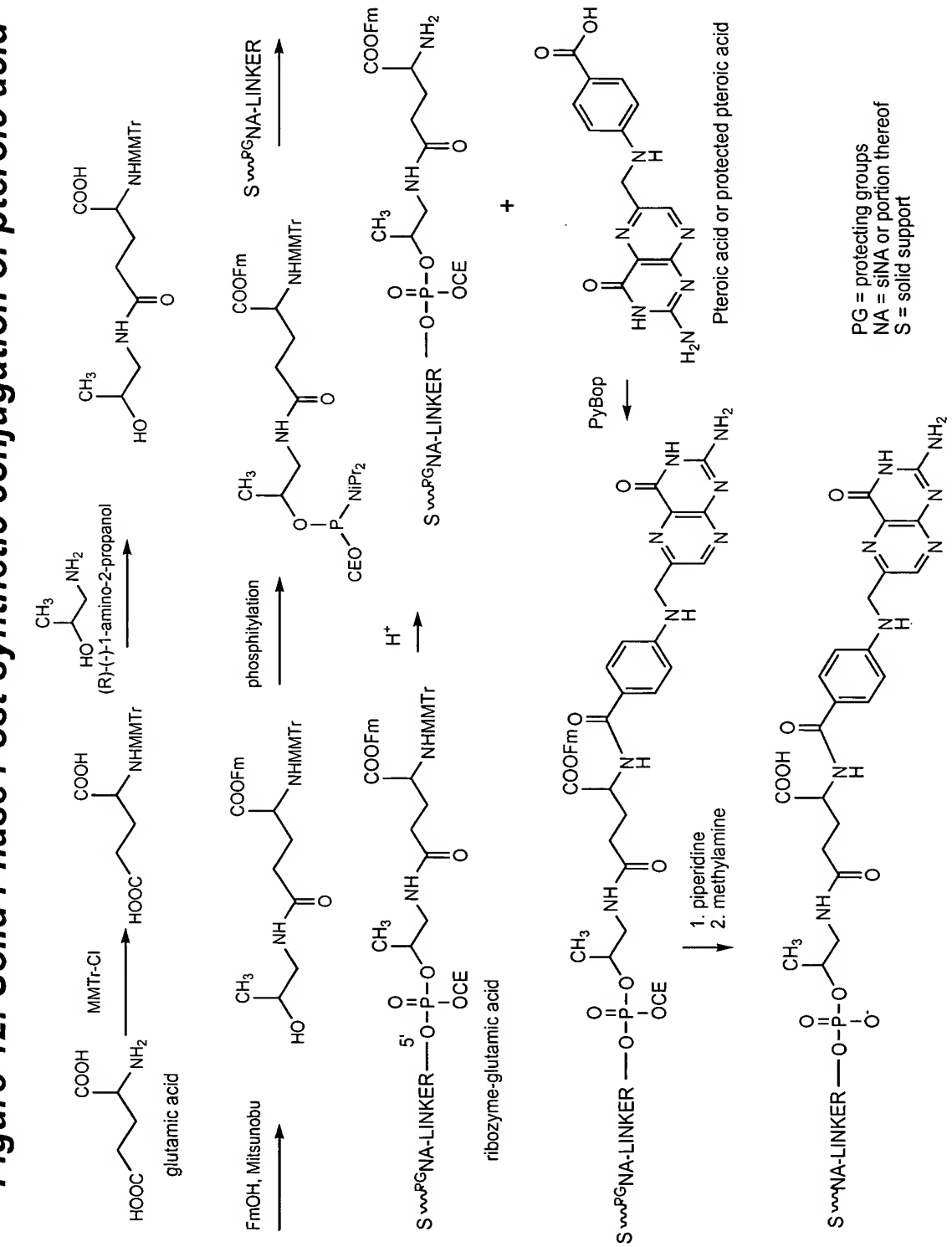
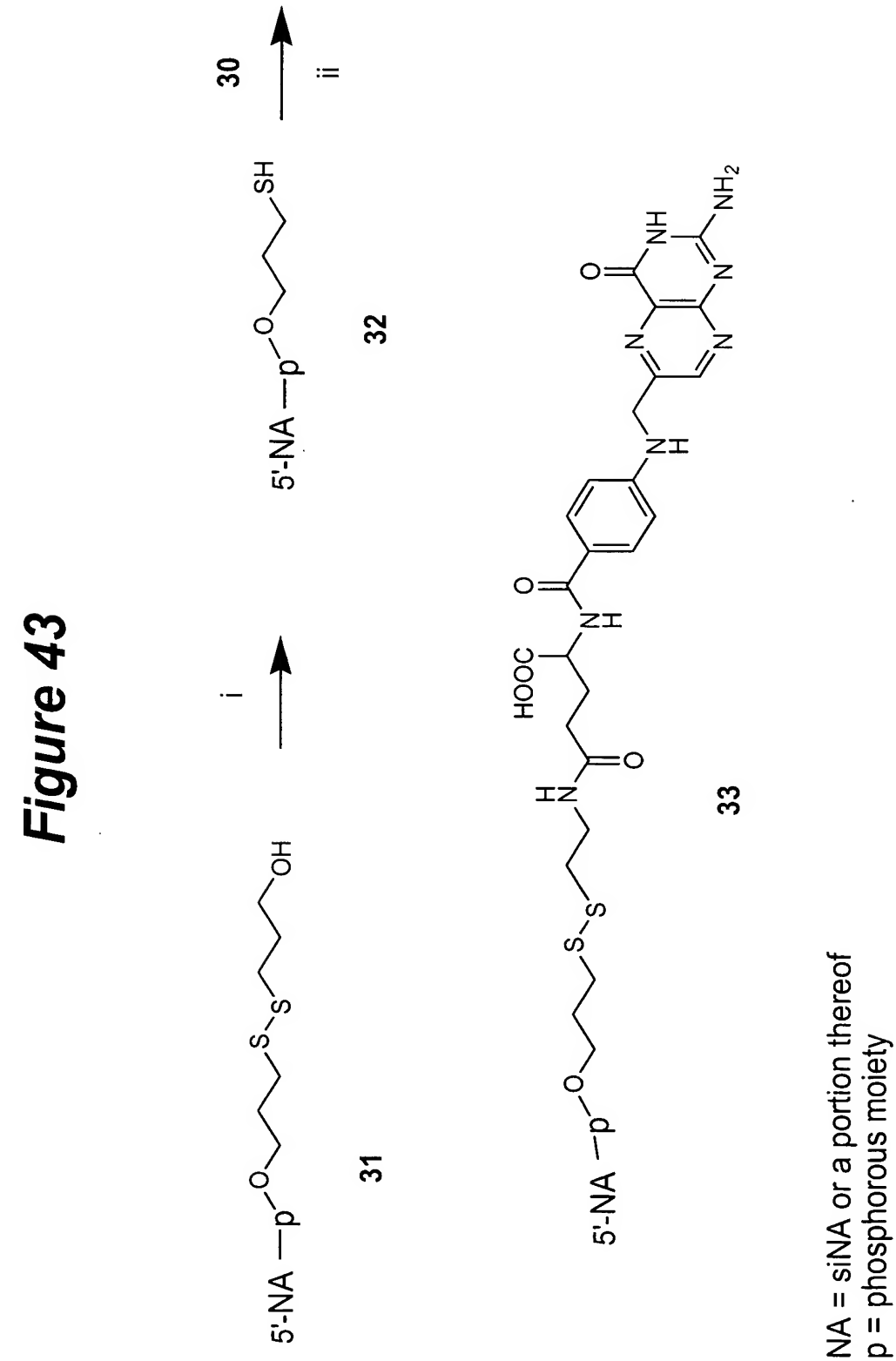
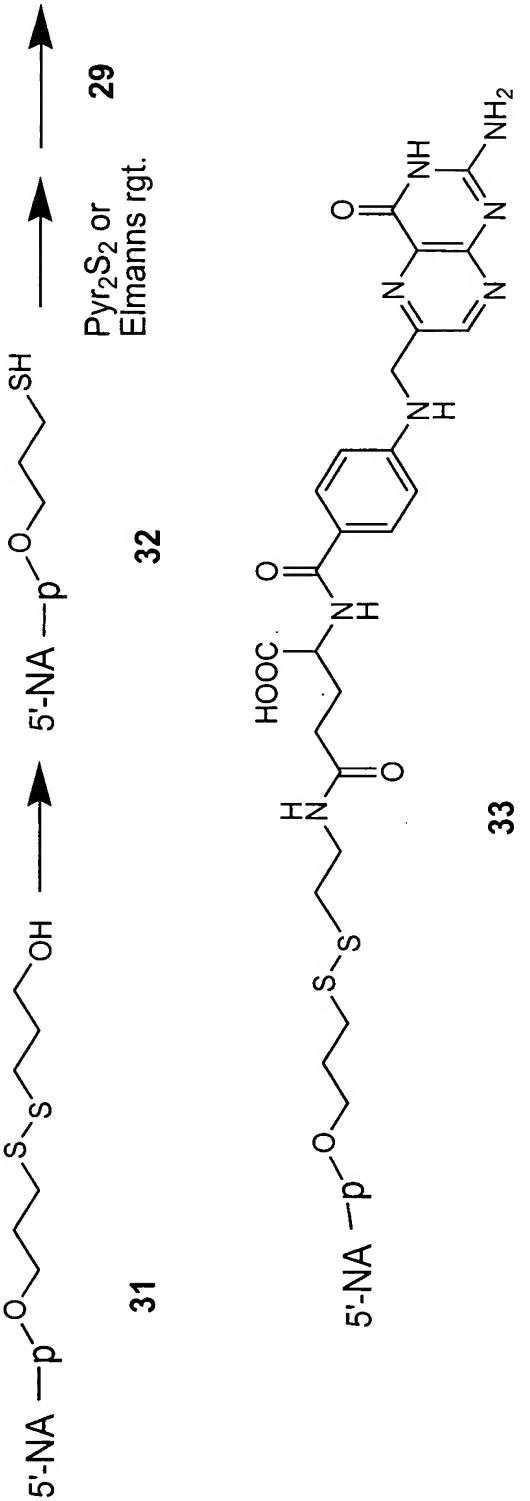


Figure 43



NA = siNA or a portion thereof
p = phosphorous moiety

Figure 44



NA = siNA or a portion thereof
p = phosphorous moiety

Figure 45: Solid Phase Post-synthetic conjugation of pteroic acid

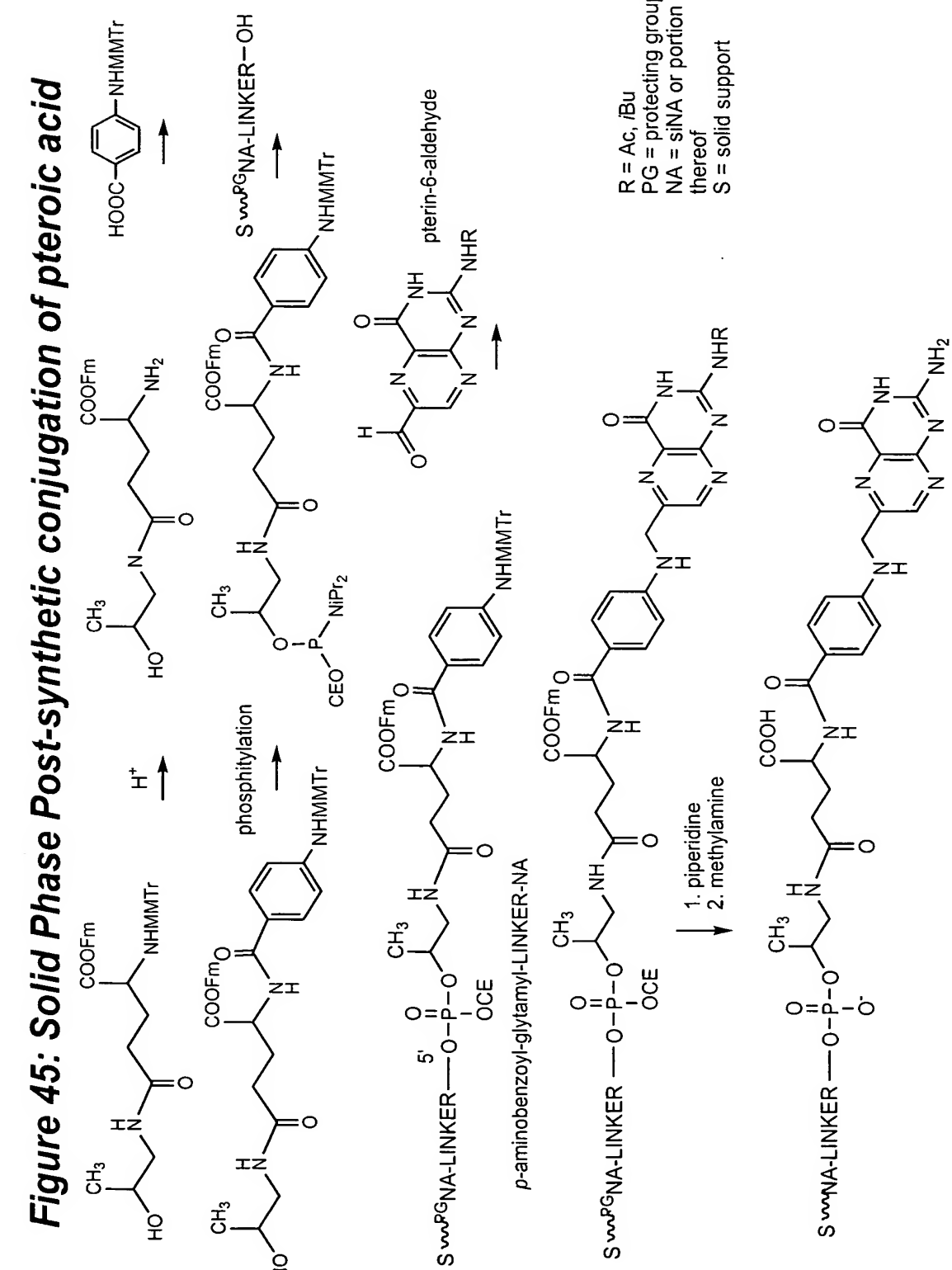
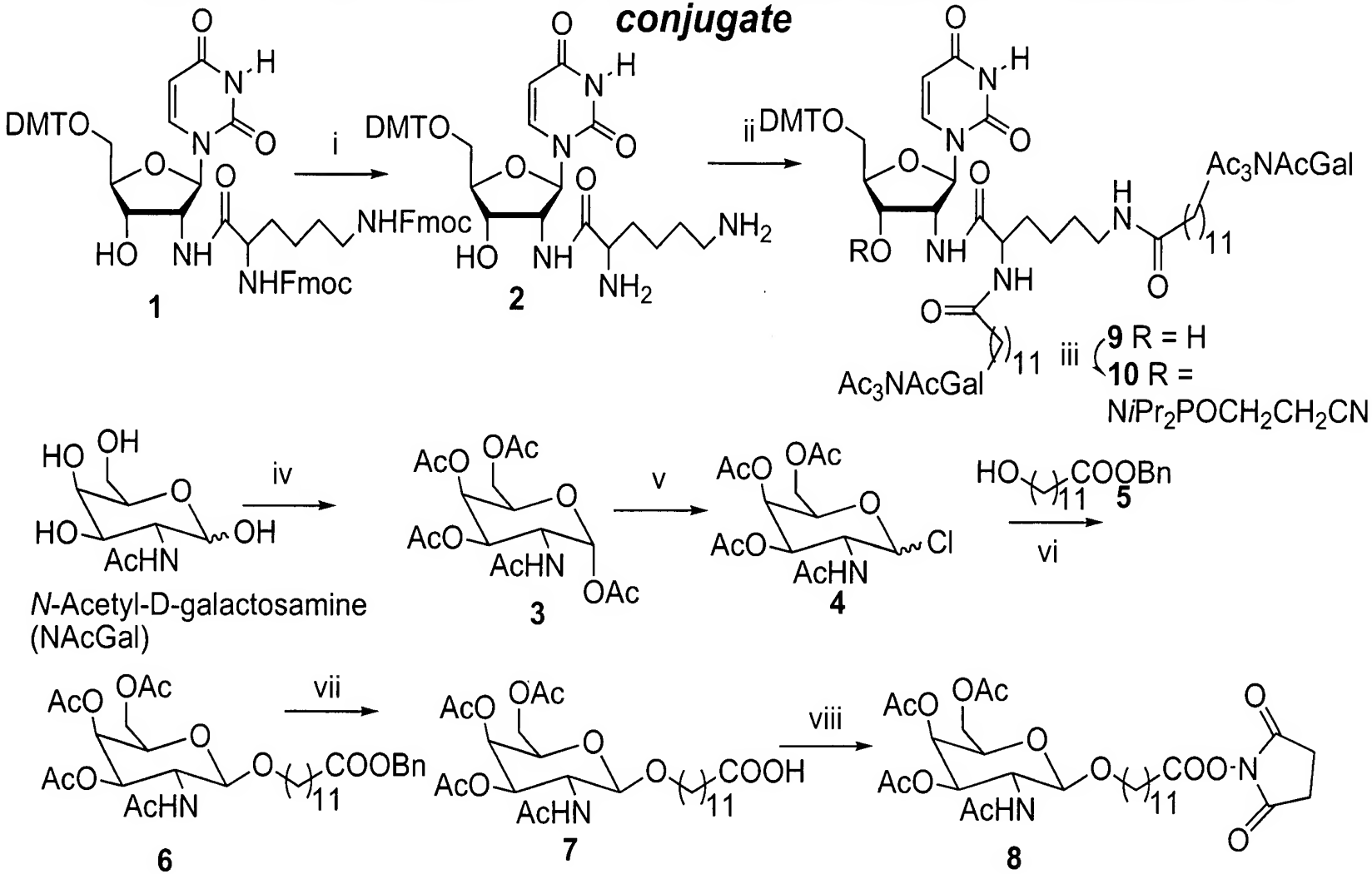
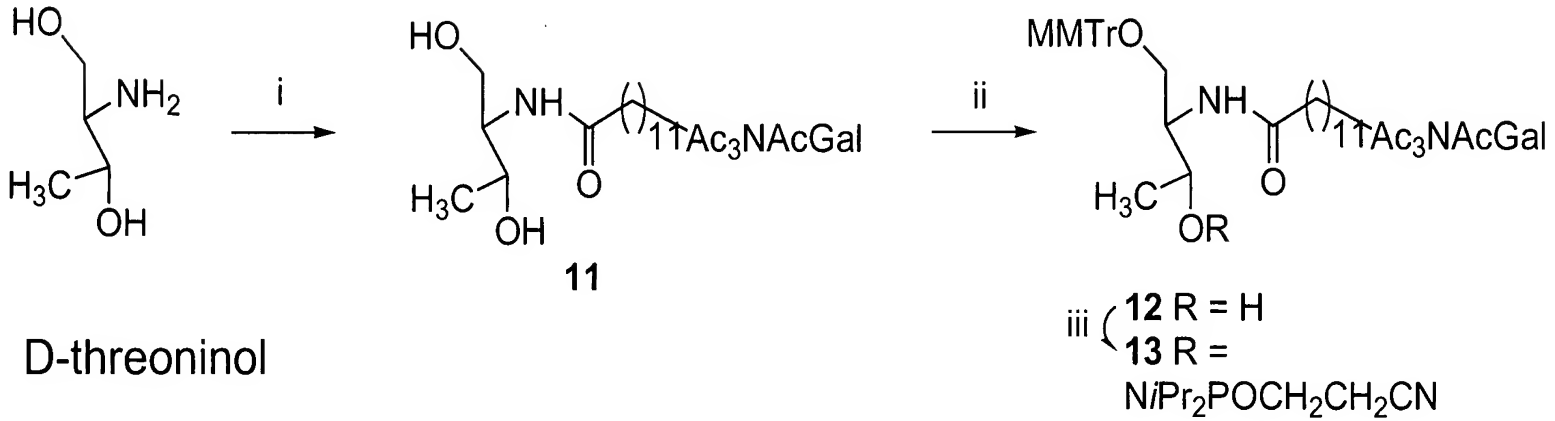


Figure 46: Synthesis of N-acetyl-D-galactosamine-2'-aminouridine conjugate



Reagents and Conditions: (i) diethylamine, DMF, (ii) **8**, diisopropylethylamine, DMF, (iii) 2-cyanoethyl *N,N*-diisopropylchlorophosphoramidite, 1-methylimidazole, DIPEA, CH_2Cl_2, (iv) Ac_2O, TEA, CH_3CN, (v) HCl, Ac_2O, (vi) $Hg(CN)_2$, MS 4A, CH_3NO_2-toluene 1:1, (vii) H_2, 5% Pd-C, ethanol, (viii) *N*-hydroxysuccinimide, DCC, THF.

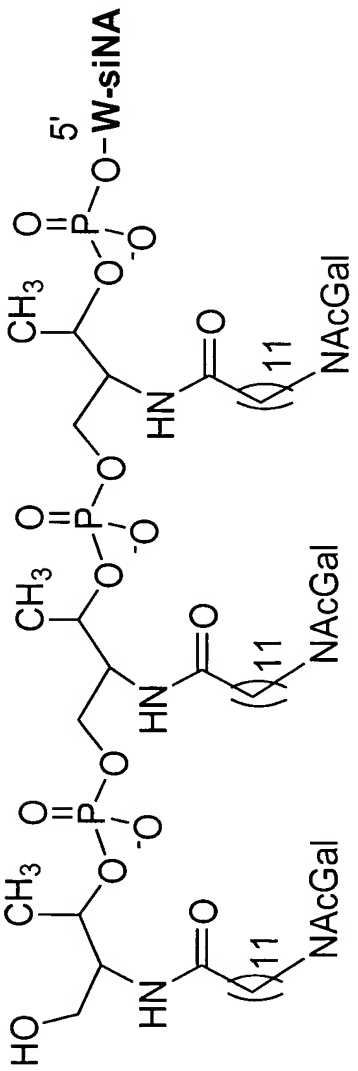
Figure 47: Synthesis of N-acetyl-D-galactosamine-D-threoninol conjugate



D-threoninol

Reagents and Conditions: (i) **7**, DCC, *N*-hydroxysuccinimide, (ii) MMTr-Cl, pyridine, (iii) 2-cyanoethyl *N,N*-diisopropylchlorophosphoramidite, 1-methylimidazole, DIPEA, CH_2Cl_2.

Figure 48: Conjugation of targeting ligands to the 5'-end of a siNA molecule



N-acetyl-D-galactosamine conjugate

Figure 49: Synthesis of dodecanoic acid linker

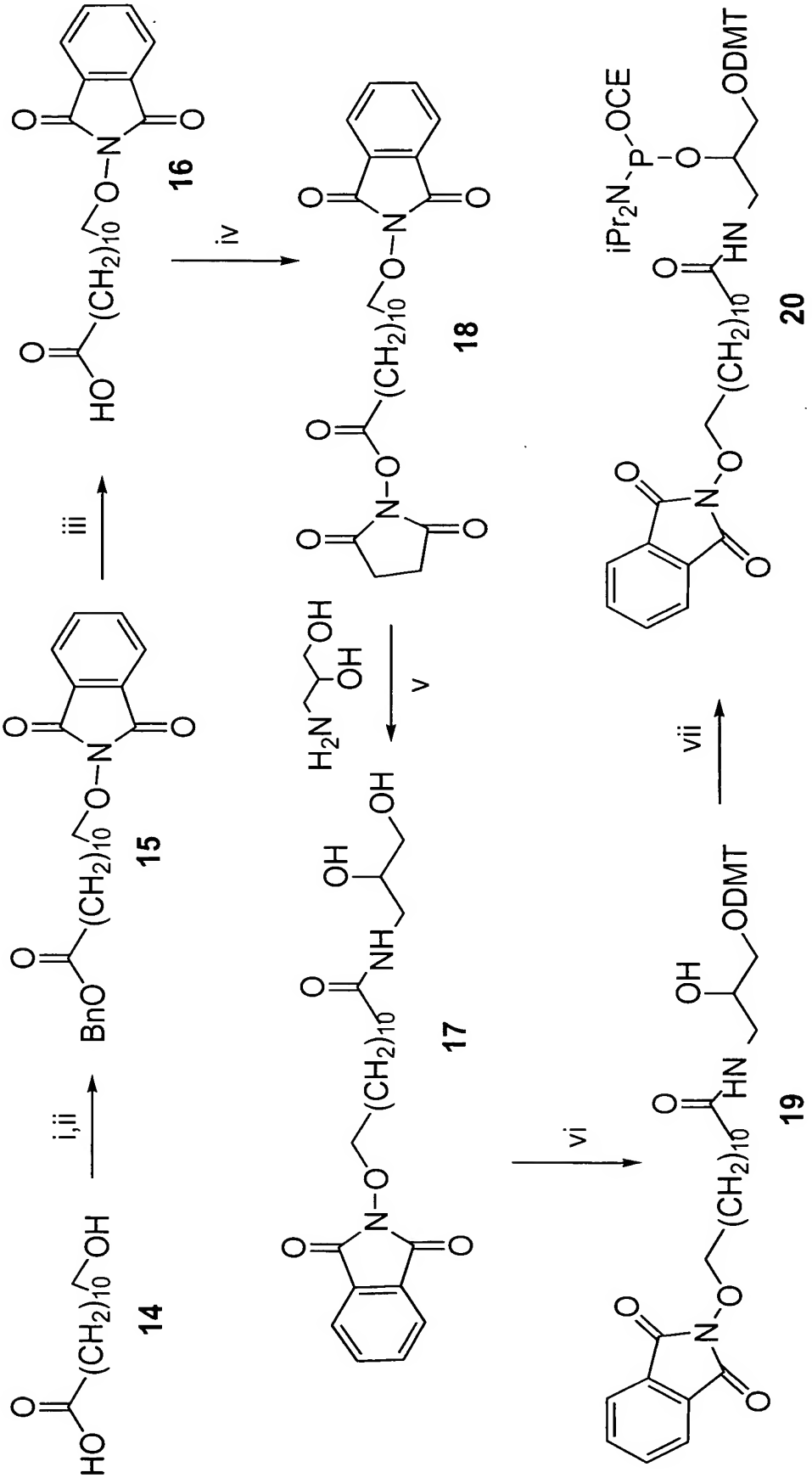


Figure 50: Oxime linked siNA/Peptide Conjugate

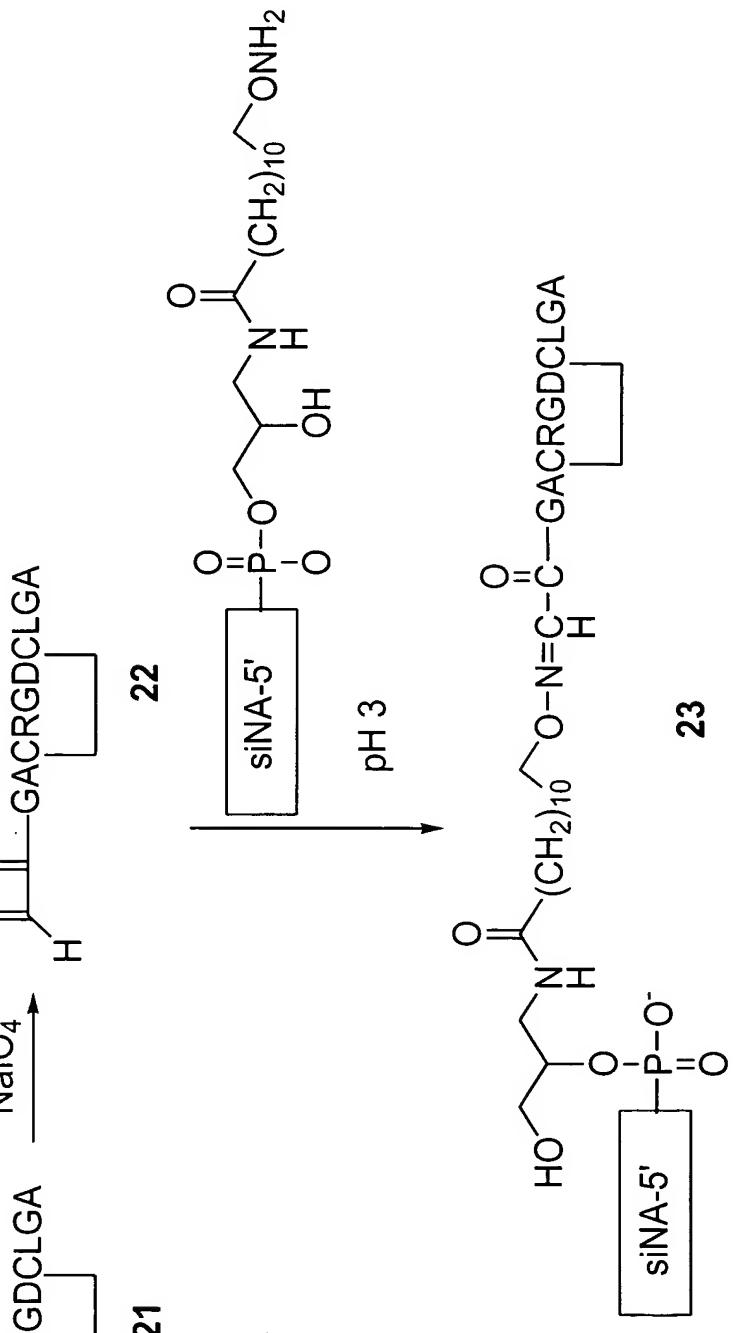
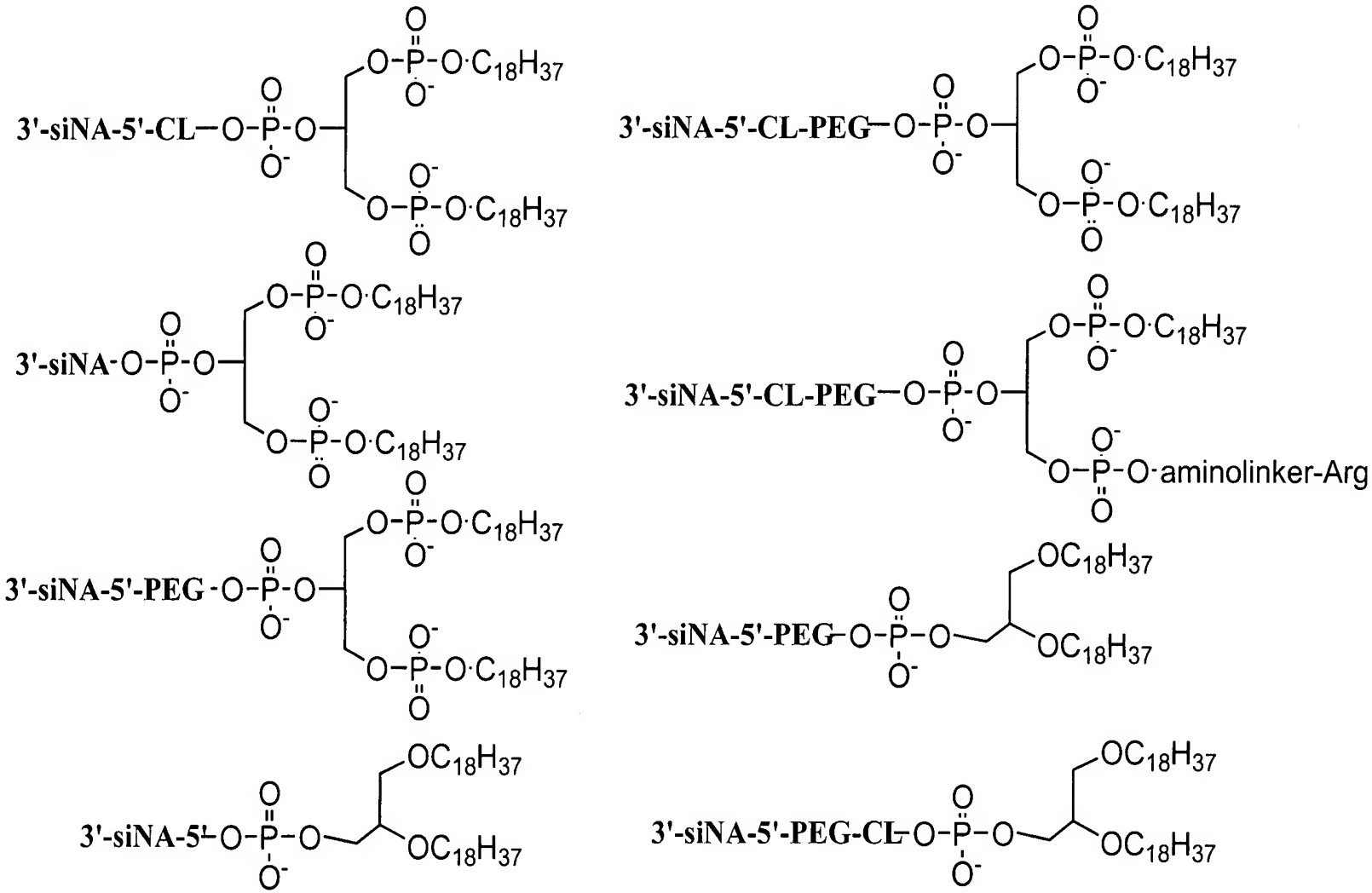
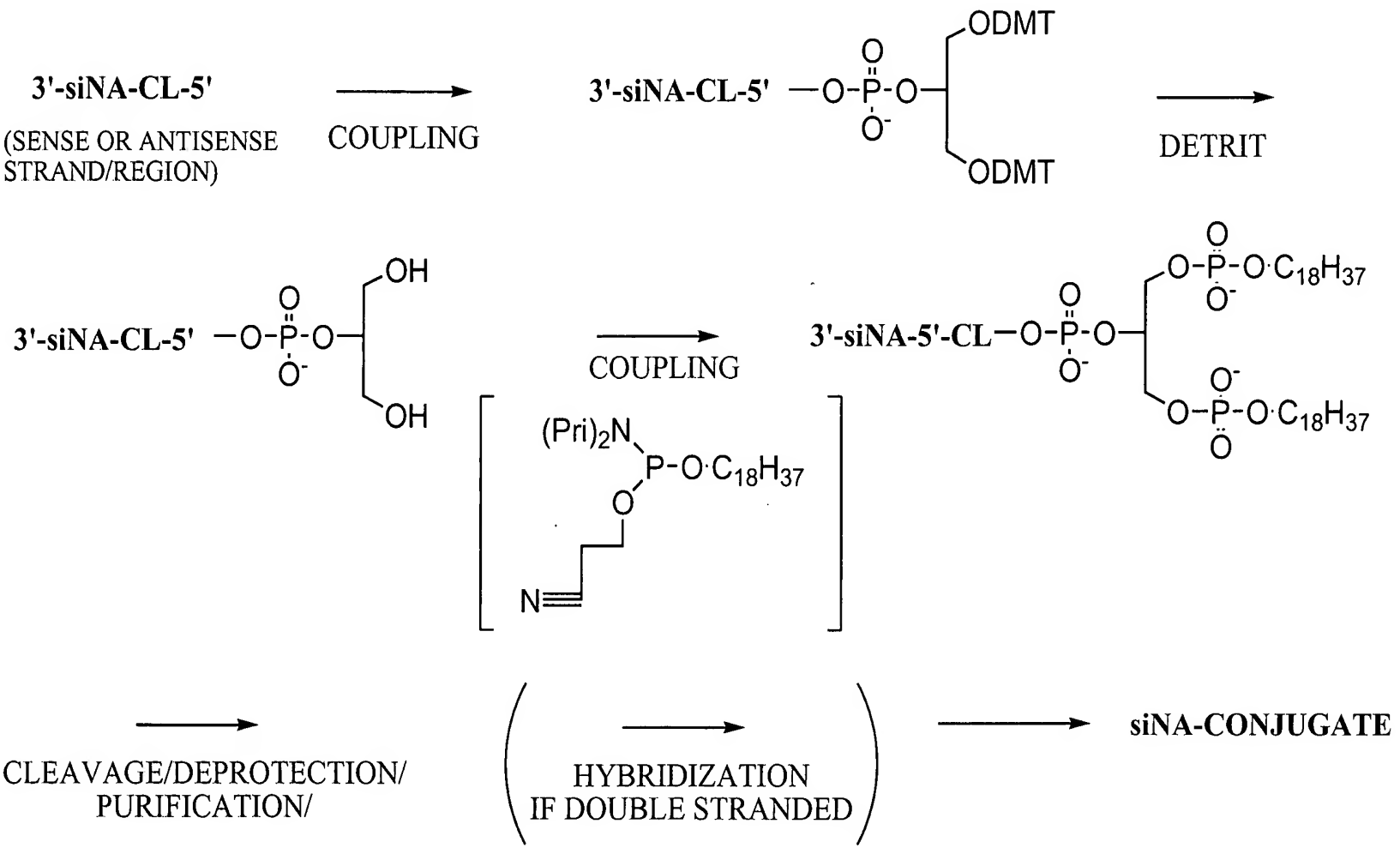


Figure 51: siNA/Phospholipid Conjugates



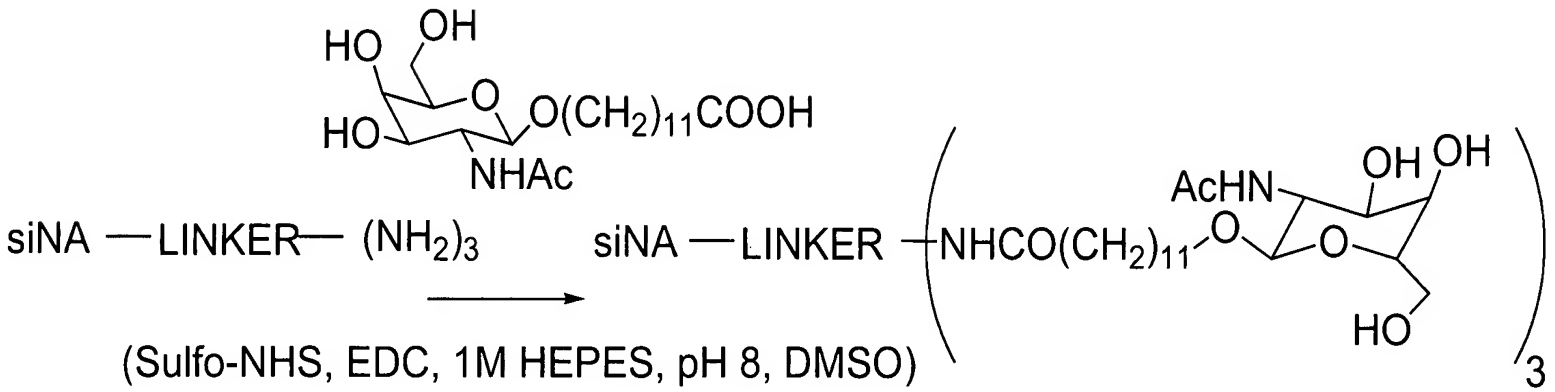
PEG=polyethylene glycol
CL=cleavable linker (e.g. A-dT, C-dT)
siNA= short interfering nucleic acid molecule or a portion thereof

Figure 52: siNA Phospholipid Conjugate

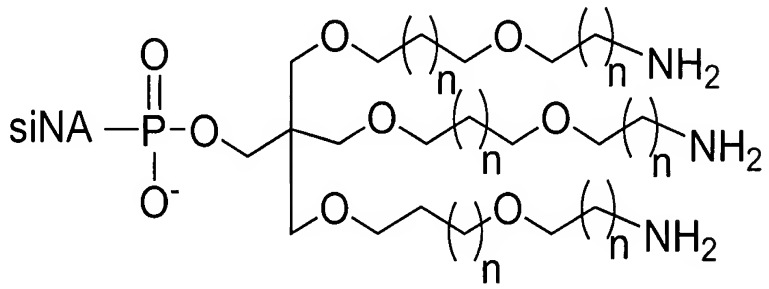


CL = CLEAVABLE LINKER, E.G. ADENOSINE-THYMIDINE DIMER THAT IS OPTIONALLY PRESENT

Figure 53: siNA-NAcGalactosamine post-synthetic coupling



FOR EXAMPLE: OLIGO-LINKER =



Where n is an integer from 1 to 20

Figure 54: siNA Cholesterol Conjugate

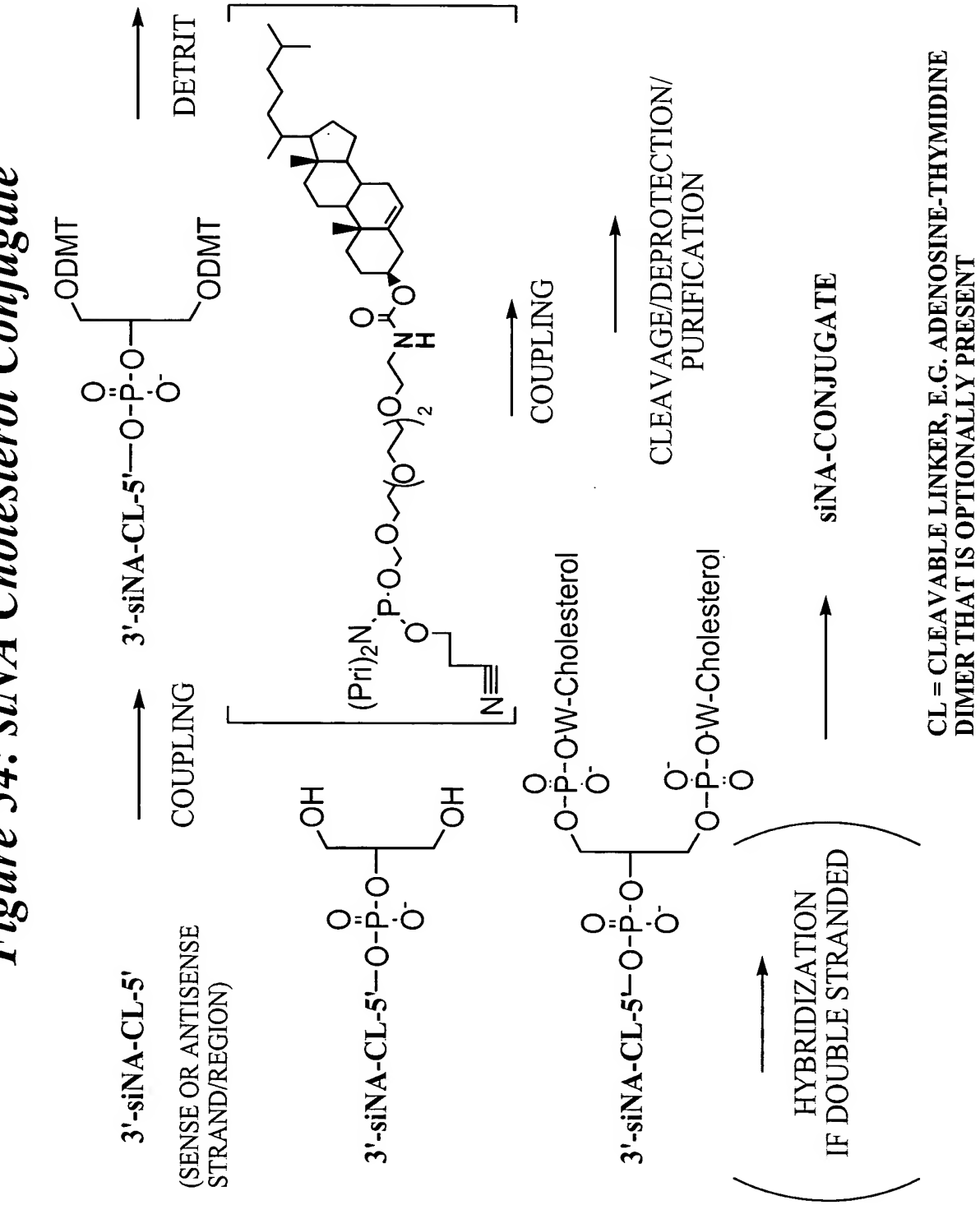
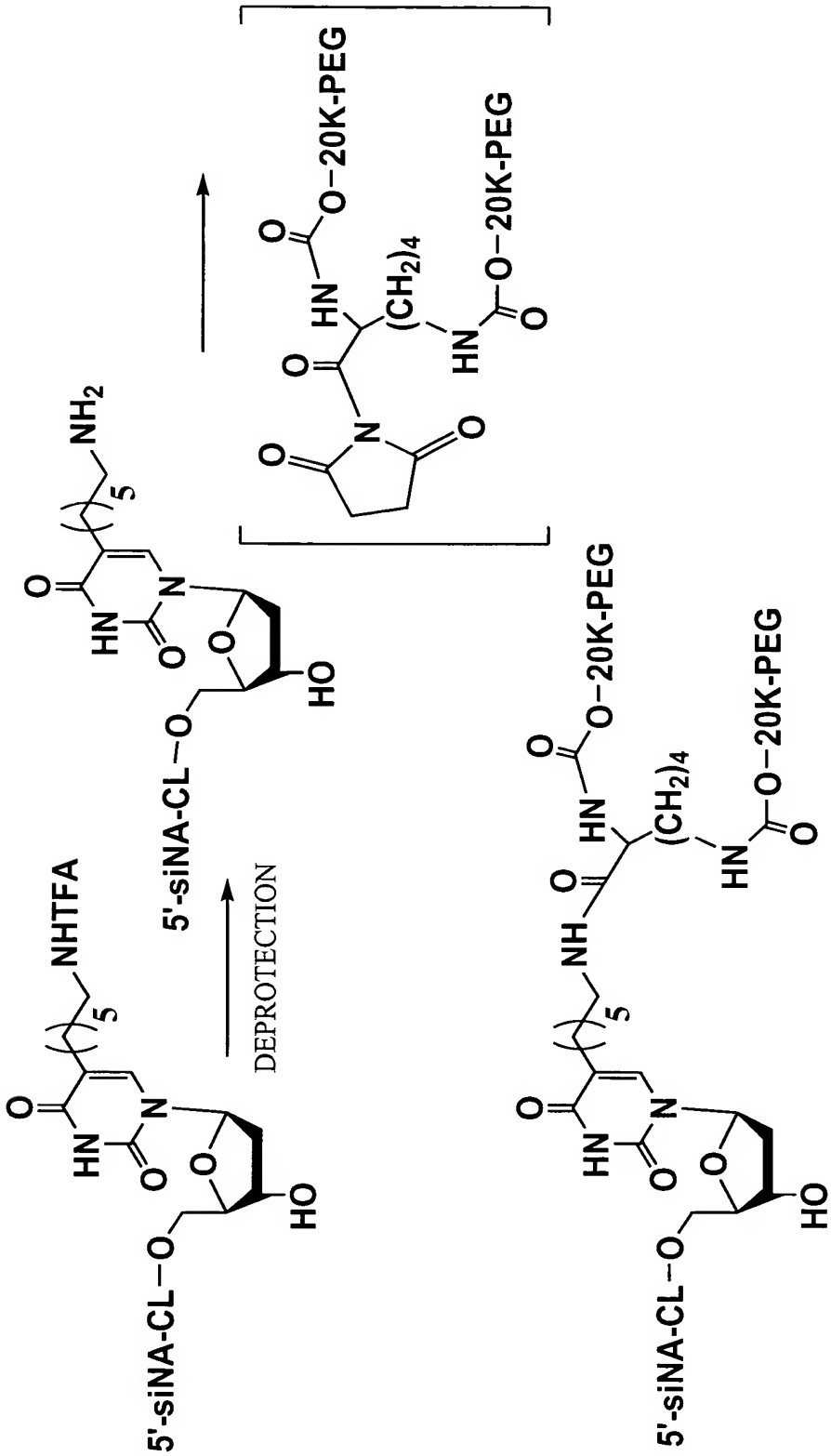


Figure 55: siNA 3'-PEG Conjugate



CL = CLEAVABLE LINKER, E.G. ADENOSINE-THYMIDINE DIMER THAT IS OPTIONALLY PRESENT

Figure 56: siNA 3'-Cholesterol Conjugate

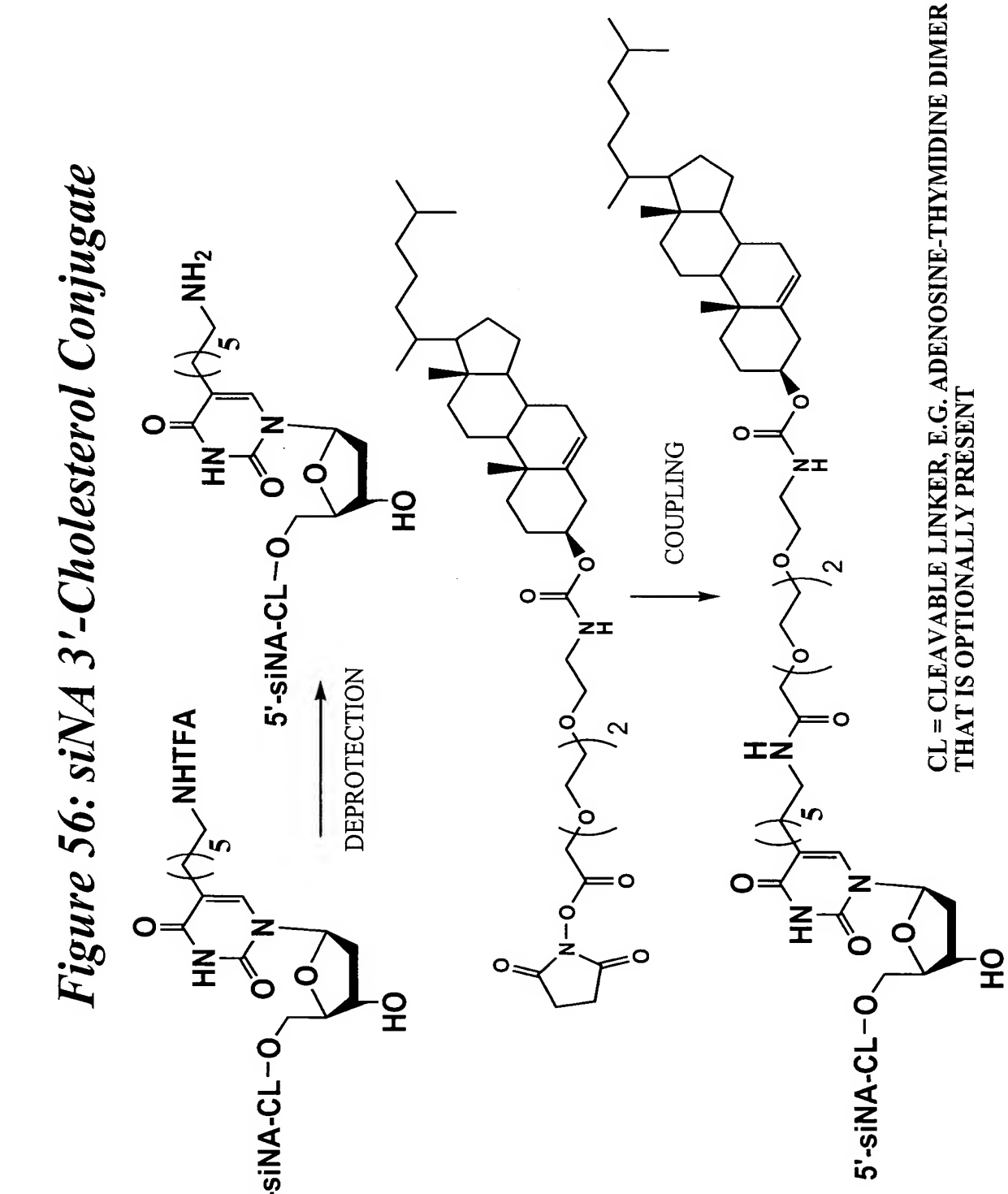
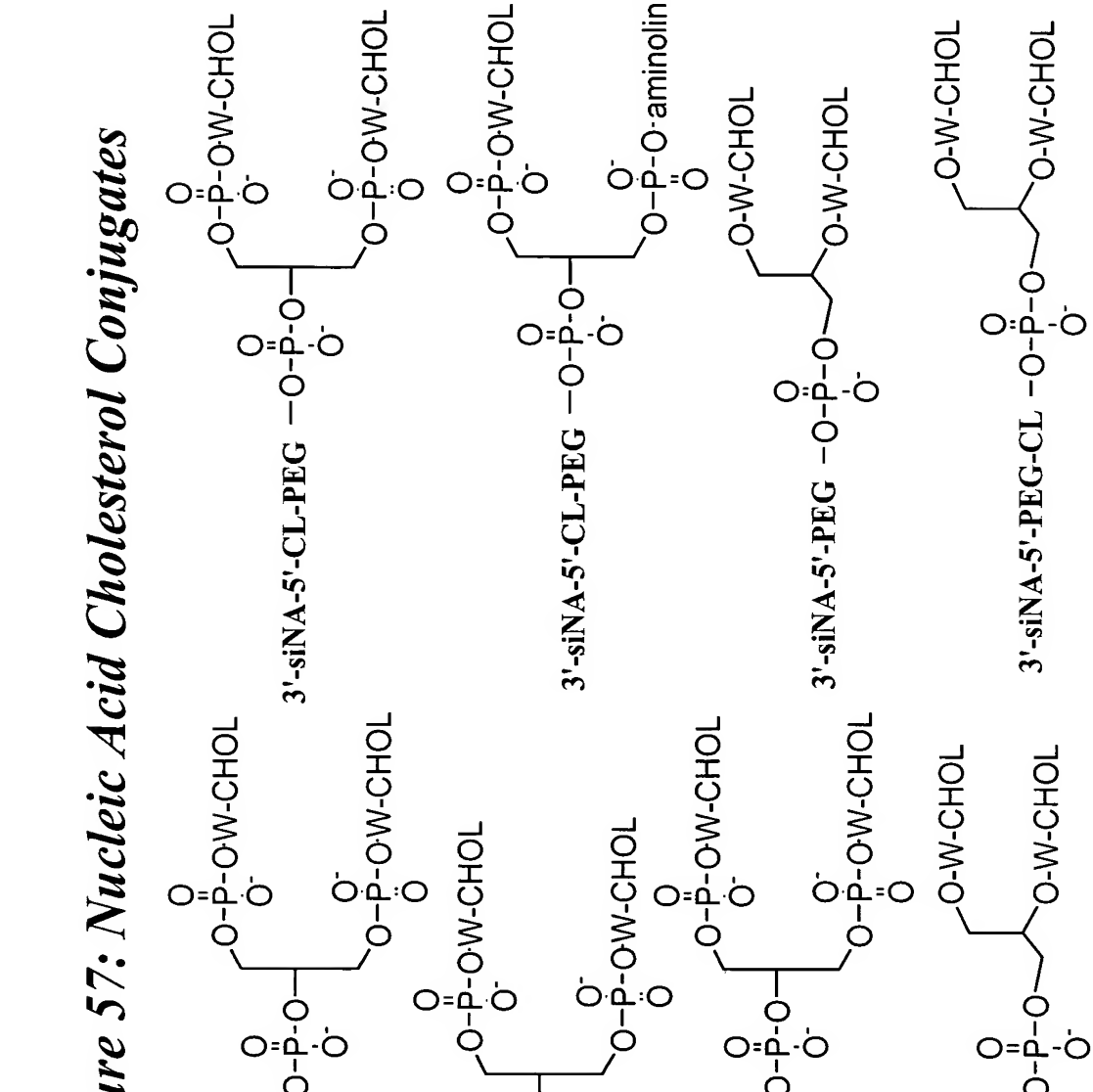
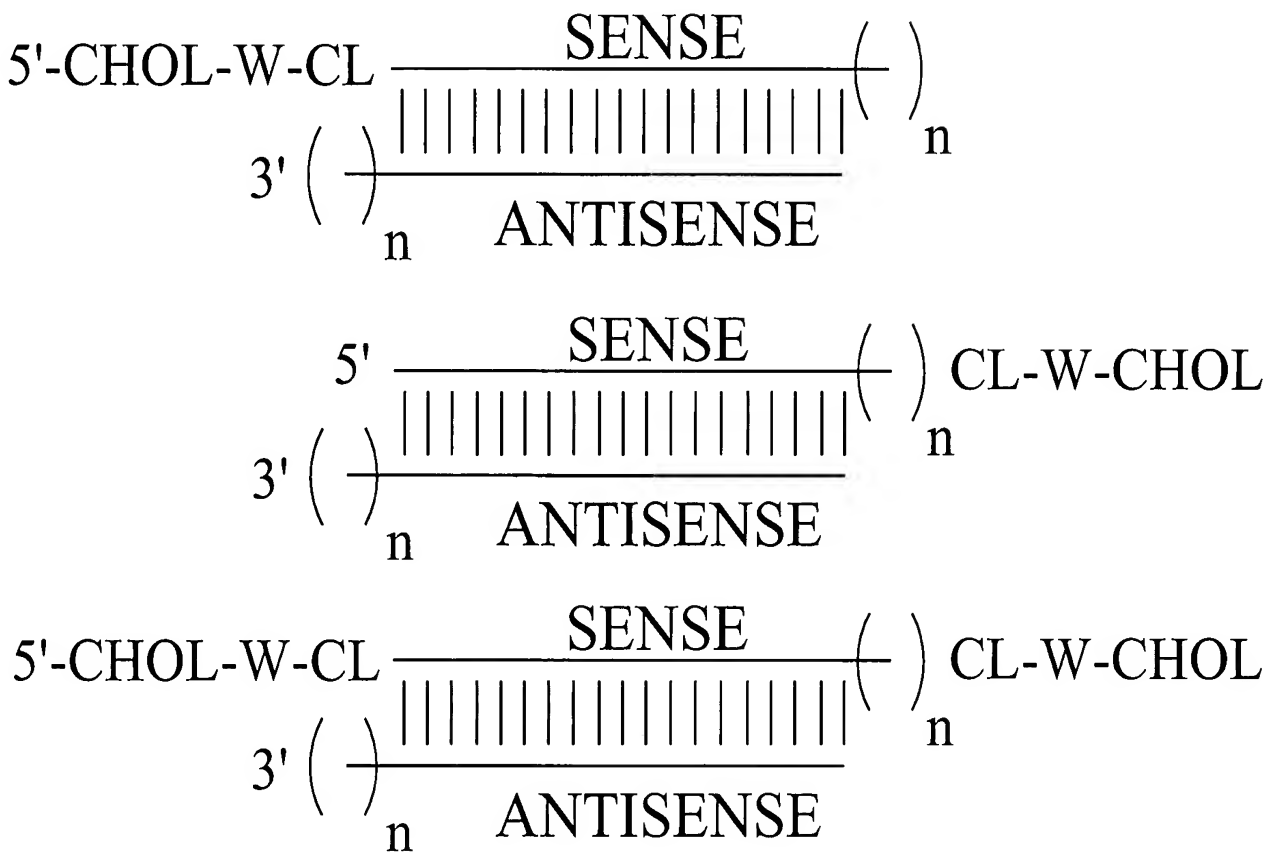


Figure 57: Nucleic Acid Cholesterol Conjugates



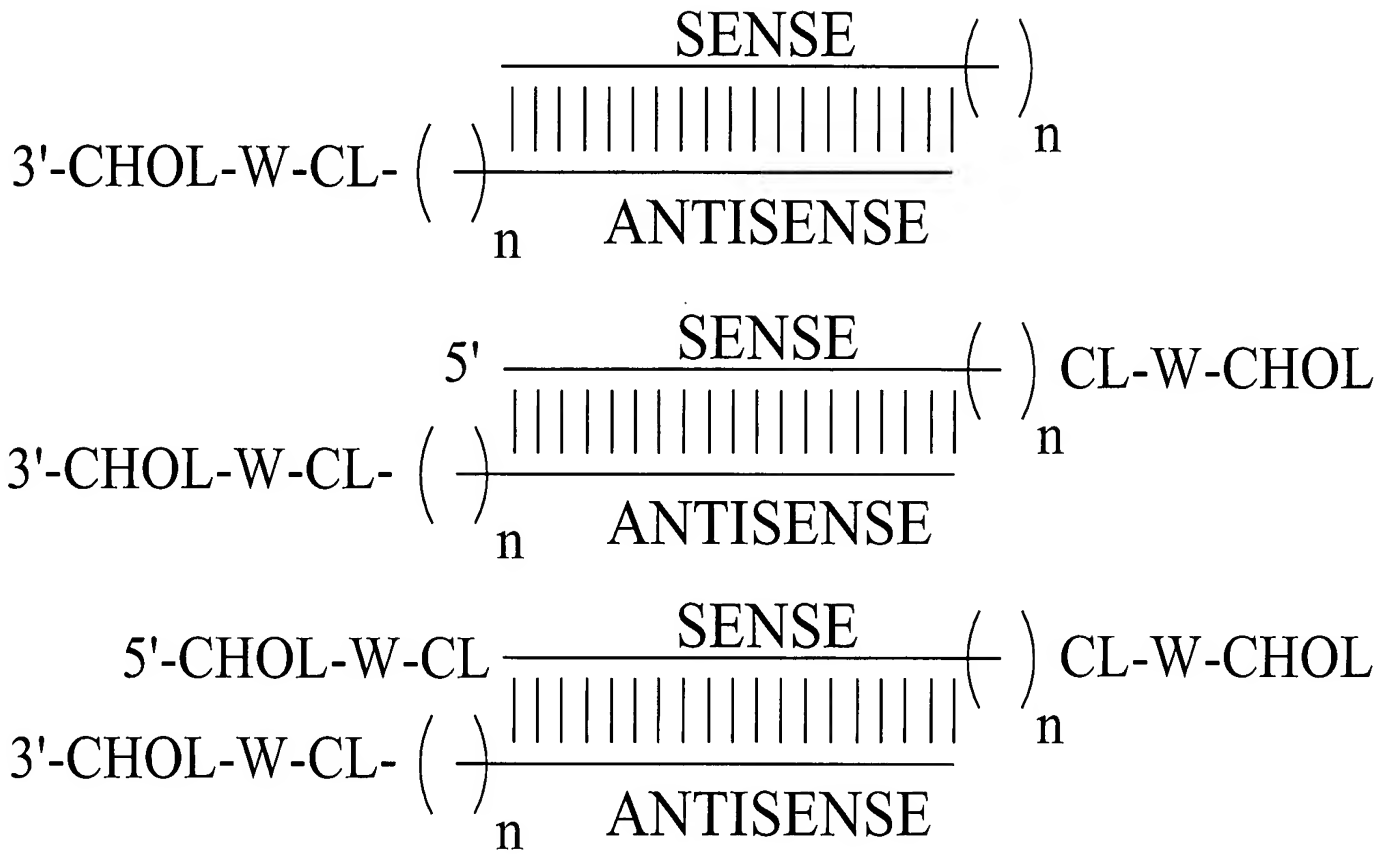
PEG=polyethylene glycol
CL=cleavable linker (e.g. A-dT, C-dT)
siNA= short interfering nucleic acid molecule or a portion thereof
CHOL=cholesterol or an analog or metabolite thereof
W= linker molecule (see for example Formulae 109 or 112)

Figure 58: siNA Cholesterol Conjugates



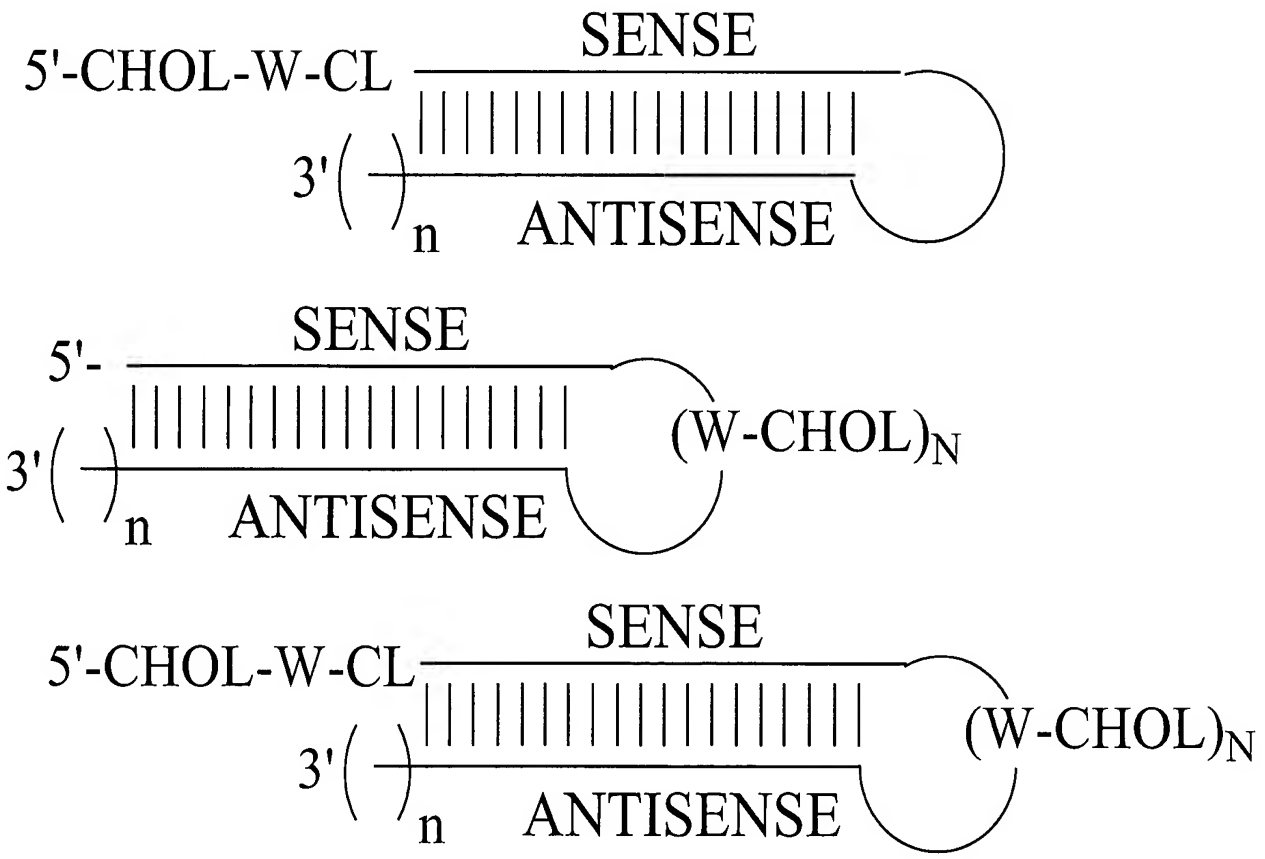
CL=cleavable linker (e.g. A-dT, C-dT) that is optionally present
CHOL=cholesterol or an analog or metabolite thereof
W= linker molecule (see for example Formulae 107, 108, 109 or 115)
n = integer, e.g. 1, 2, or 3

Figure 59: siNA Cholesterol Conjugates



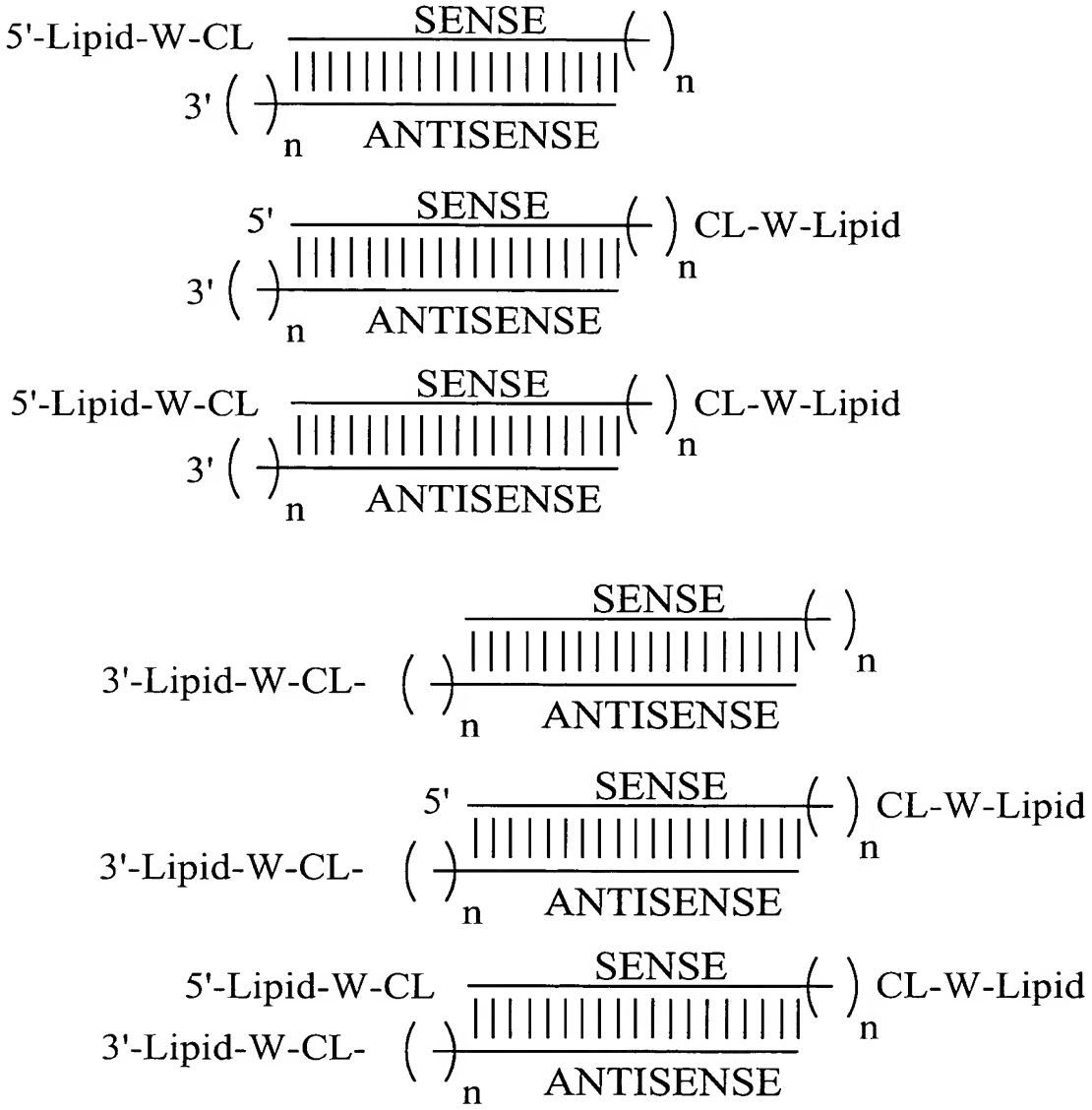
CL=cleavable linker (e.g. A-dT, C-dT) that is optionally present
CHOL=cholesterol or an analog or metabolite thereof
W= linker molecule (see for example Formulae 107, 108, 109 or 115)
n = integer, e.g. 1, 2, or 3

Figure 60: siNA Cholesterol Conjugates



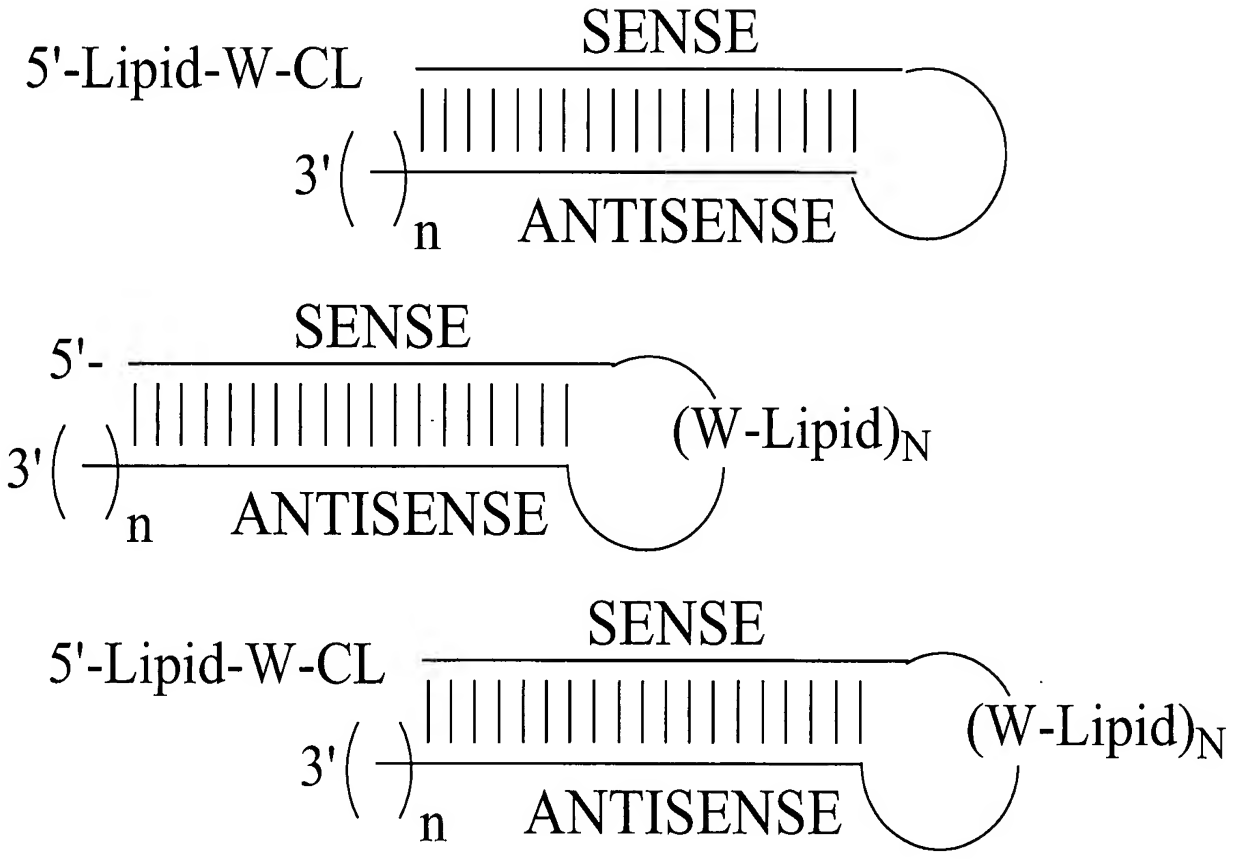
CL=cleavable linker (e.g. A-dT, C-dT) that is optionally present
CHOL=cholesterol or an analog or metabolite thereof
W= linker molecule (see for example Formulae 107, 108, 109 or 112)
n = integer, e.g. 1, 2, or 3
N=integer, e.g. 1, 2, 3, or 4

Figure 61: siNA Lipid Conjugates



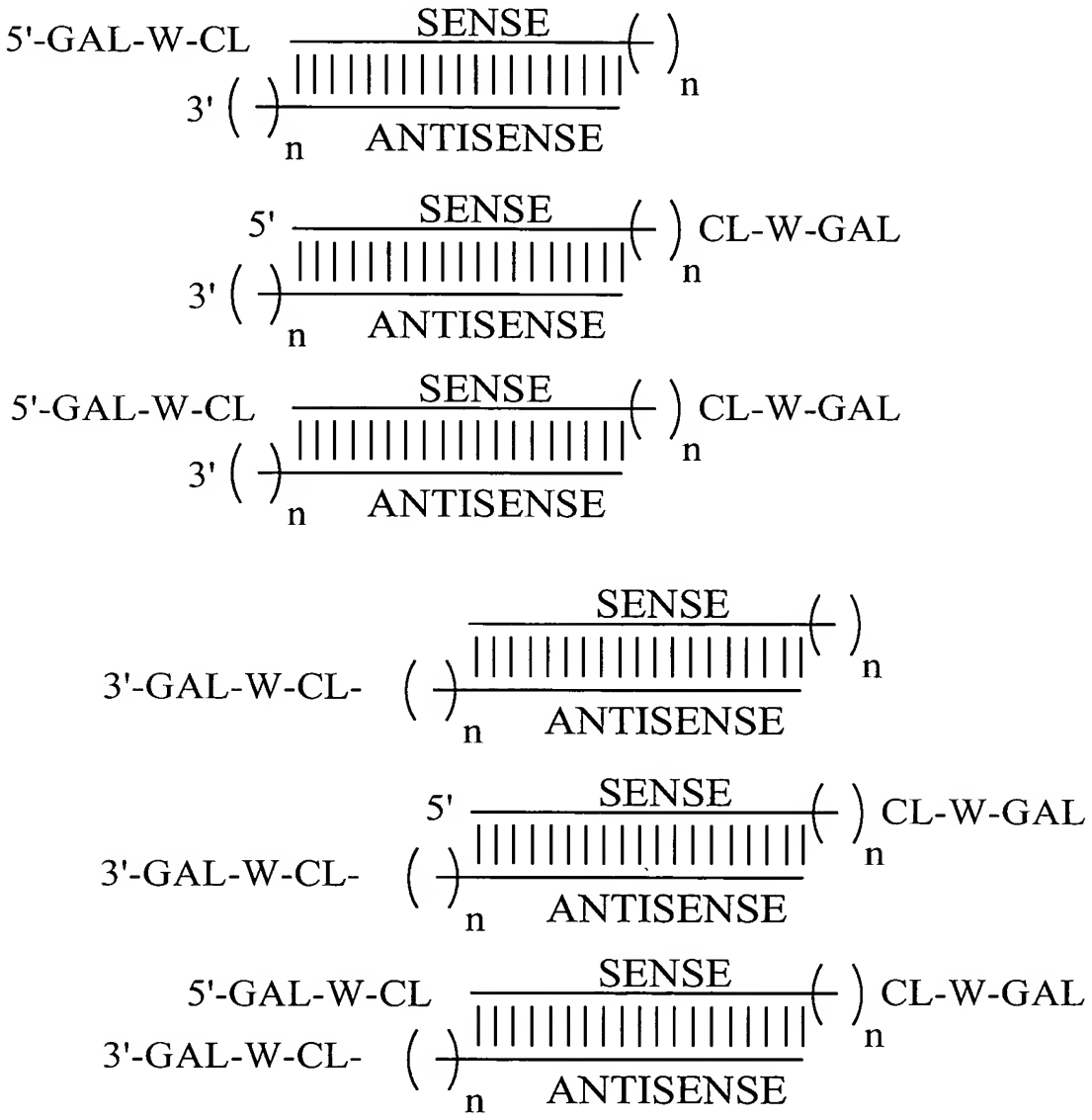
CL=cleavable linker (e.g. A-dT, C-dT) that is optionally present
Lipid=Straight chain or branched alkyl or fatty acid, e.g. $C_{18}H_{37}$
W= linker molecule (see for example Formulae 48, 49, 64, or 65)
n = integer, e.g. 1, 2, or 3

Figure 62: siNA Lipid Conjugates



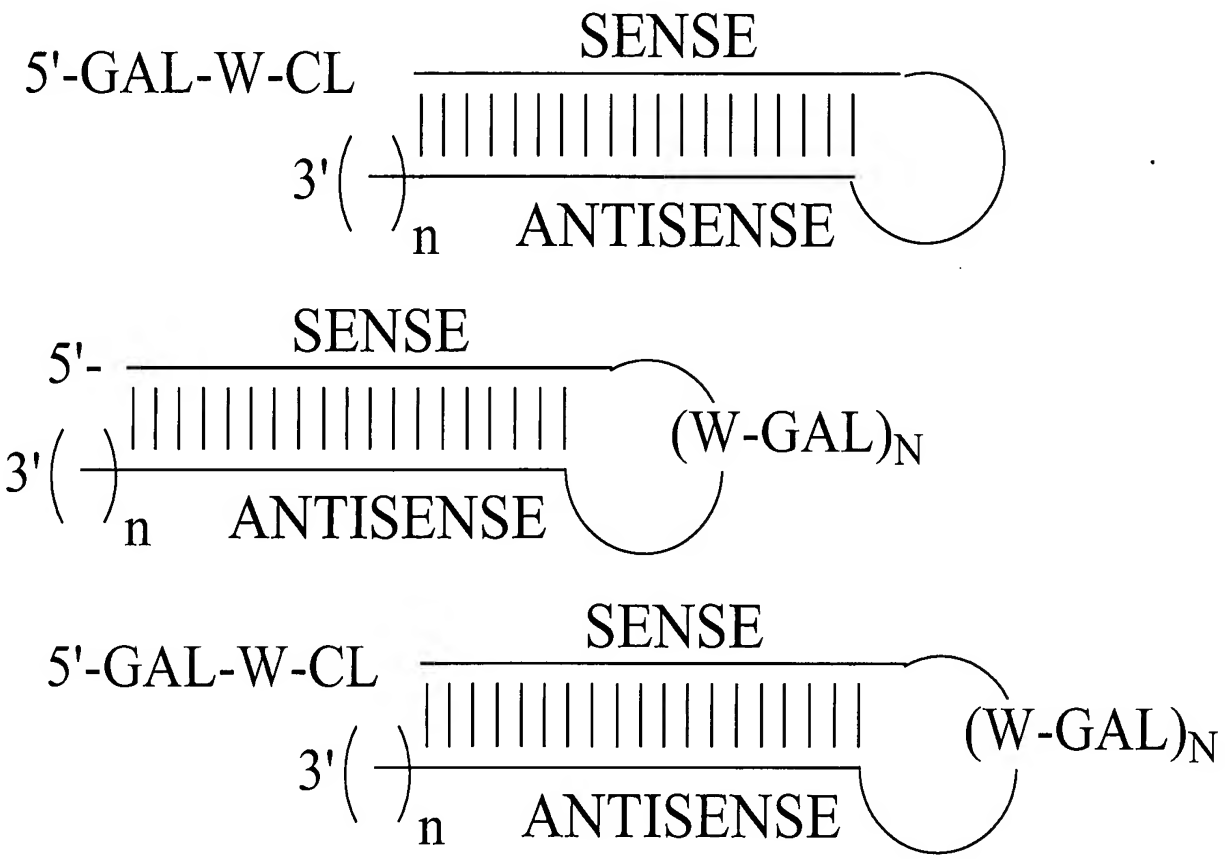
CL=cleavable linker (e.g. A-dT, C-dT) that is optionally present
Lipid=Straight chain or branched alkyl or fatty acid, e.g. $C_{18}H_{37}$
W= linker molecule (see for example Formulae 48, 49, 64, or 65)
n = integer, e.g. 1, 2, or 3
N=integer, e.g. 1, 2, 3, or 4

Figure 63: siNA Galactosamine Conjugates



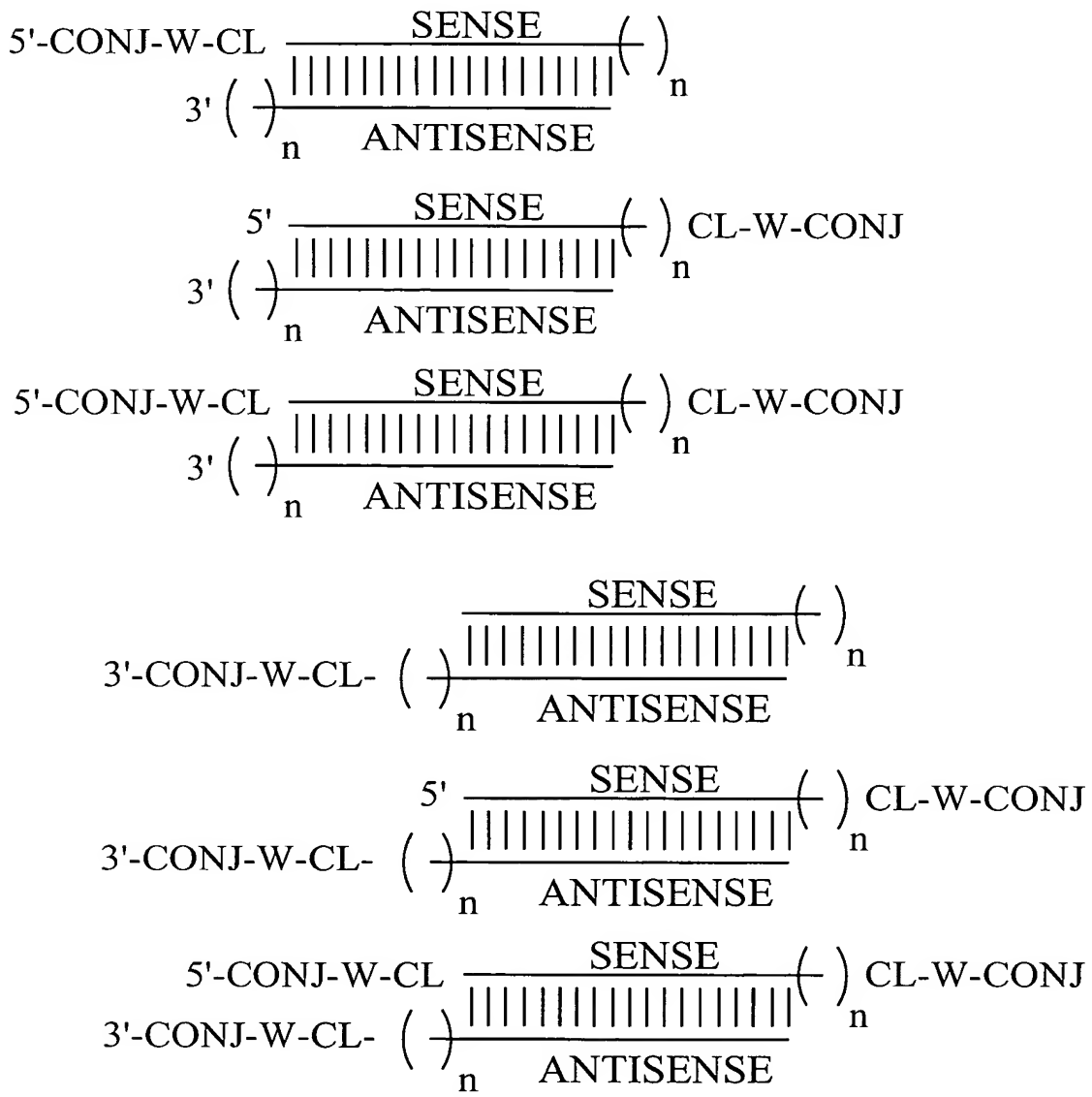
CL=cleavable linker (e.g. A-dT, C-dT) that is optionally present
GAL=GALACTOSAMINE; e.g. compounds having Formulae 51-56, 86, 92, 99, 100, 103, 105, 106
W= linker molecule (see for example Formulae 102 or 103)
n = integer, e.g. 1, 2, or 3

Figure 64: siNA Galactosamine Conjugates



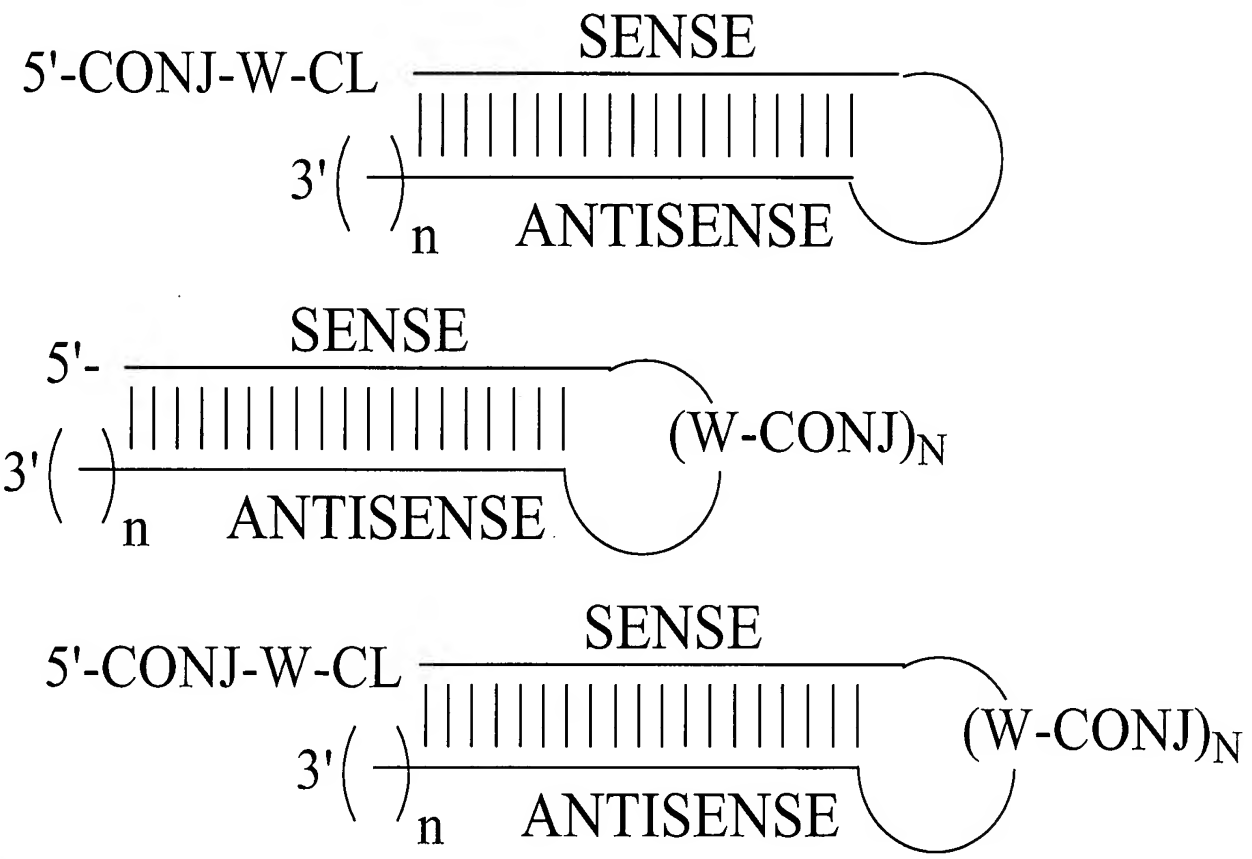
CL=cleavable linker (e.g. A-dT, C-dT) that is optionally present
GAL=GALACTOSAMINE; e.g. compounds having Formulae 51-56, 86, 92, 99, 100, 103, 105, 106
W= linker molecule (see for example Formulae 102 or 103)
n = integer, e.g. 1, 2, or 3
N=integer, e.g. 1, 2, 3, or 4

Figure 65: Generalized siNA Conjugate Design



CONJ=any biologically active molecule or conjugate as described herein
CL=cleavable linker (e.g. A-dT, C-dT) that is optionally present
W= linker molecule
n = integer, e.g. 1, 2, or 3

Figure 66: Generalized siNA Conjugate design



CONJ=any biologically active molecule or conjugate as described herein
CL=cleavable linker (e.g. A-dT, C-dT) that is optionally present
W= linker molecule
n = integer, e.g. 1, 2, or 3
N=integer, e.g. 1, 2, 3, or 4

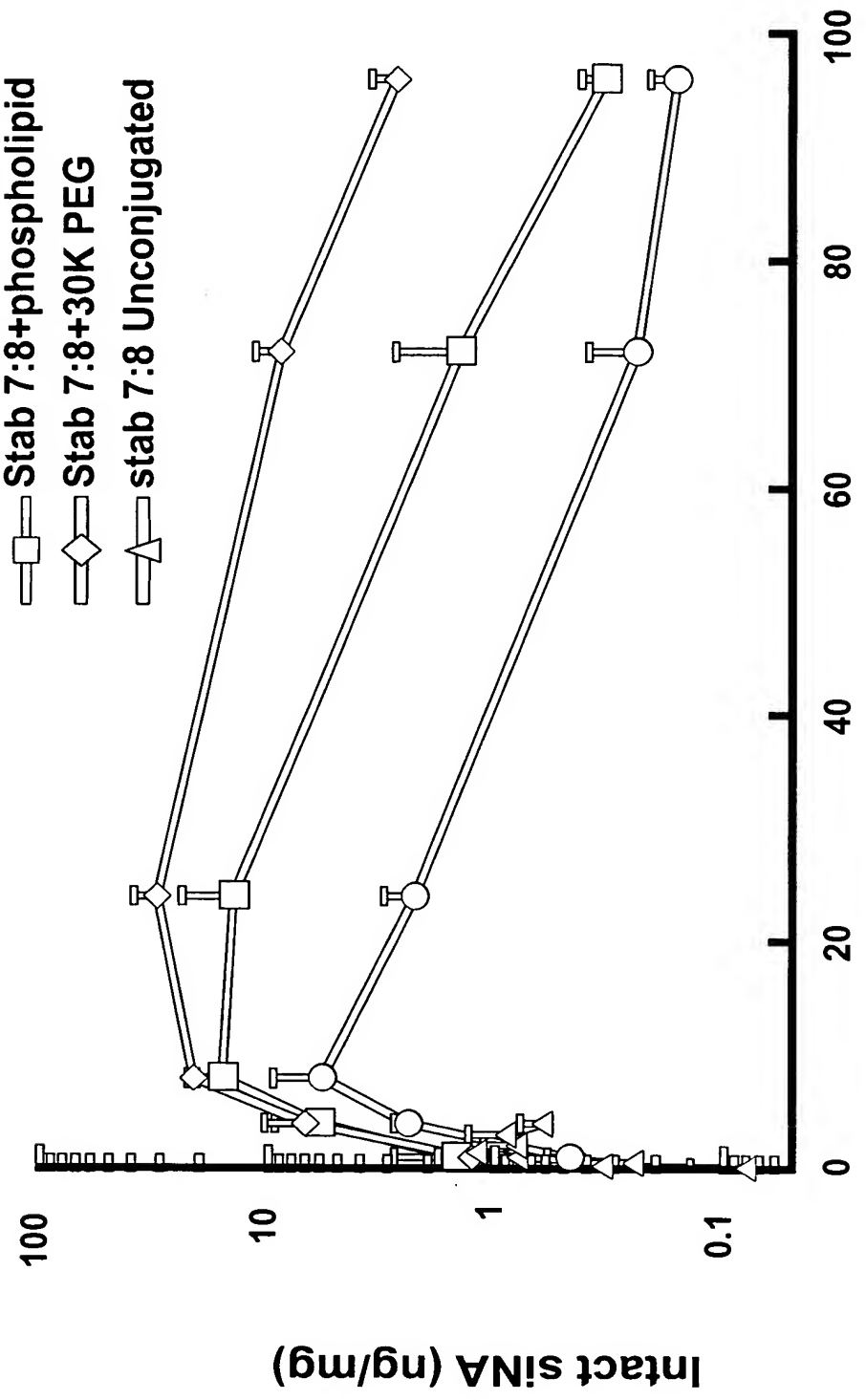


Figure 67: Distribution of Intact siNA in Liver After SC Administration of Conjugated or Unconjugated Chemistries

Figure 68: Lipid Free Delivery of HBV siNA Conjugates in Cell Culture

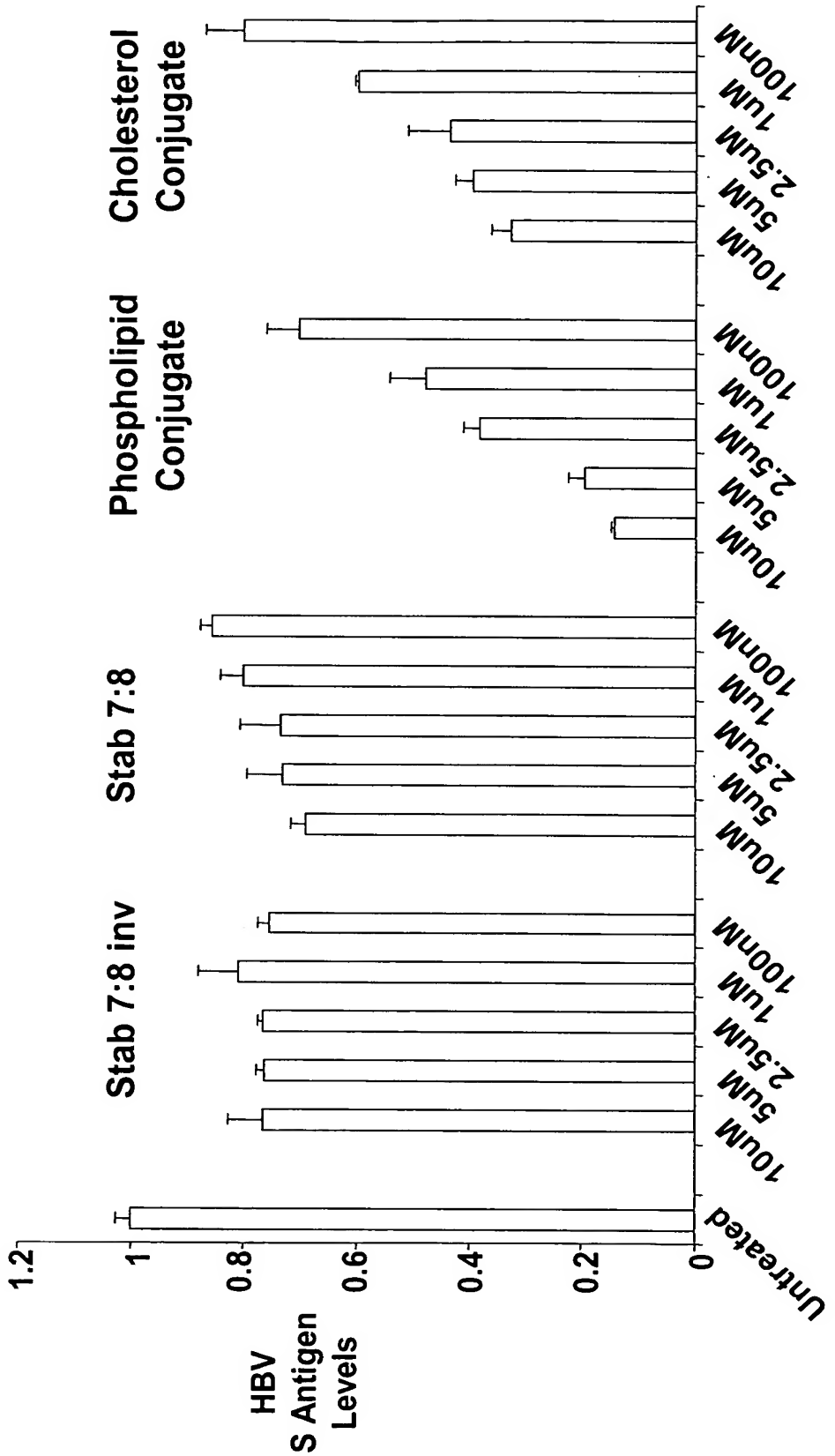


Figure 69: Scale-up of "mono" Galactosamine phosphoramidite

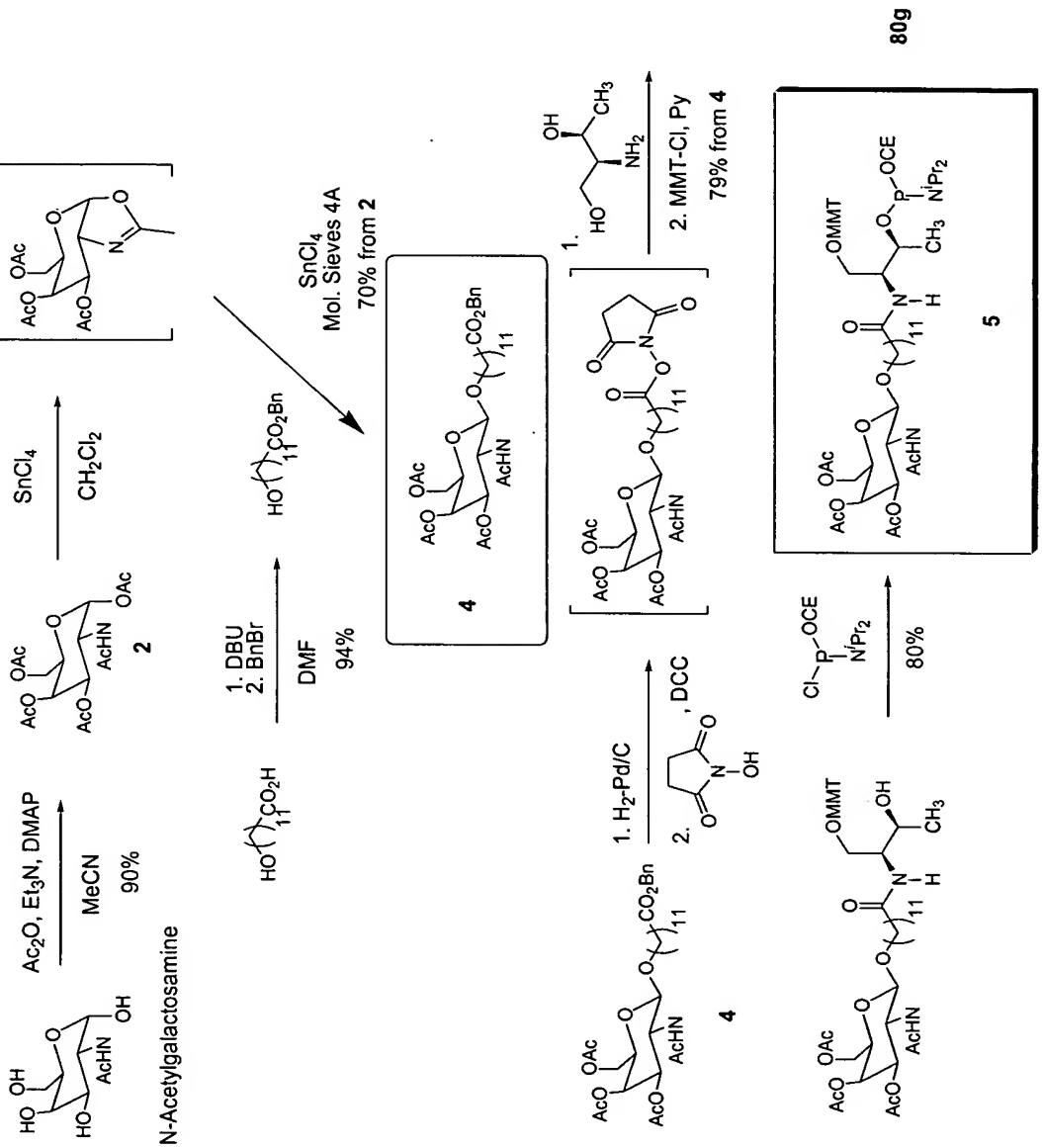


Figure 70: Synthesis of "tri" Galactosamine phosphoramidite

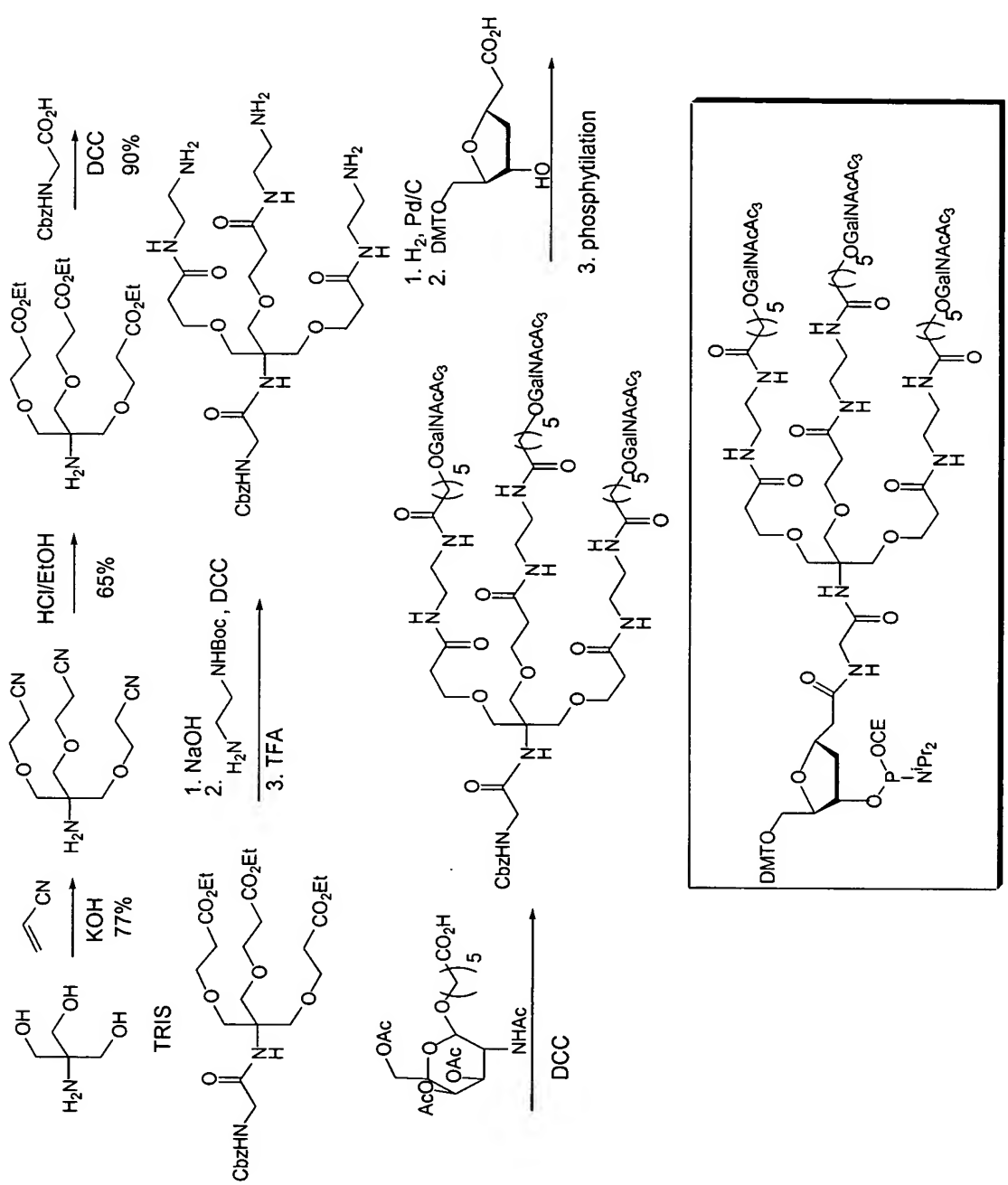


Figure 71: Synthesis of another Tri-Galactosamine Conjugate

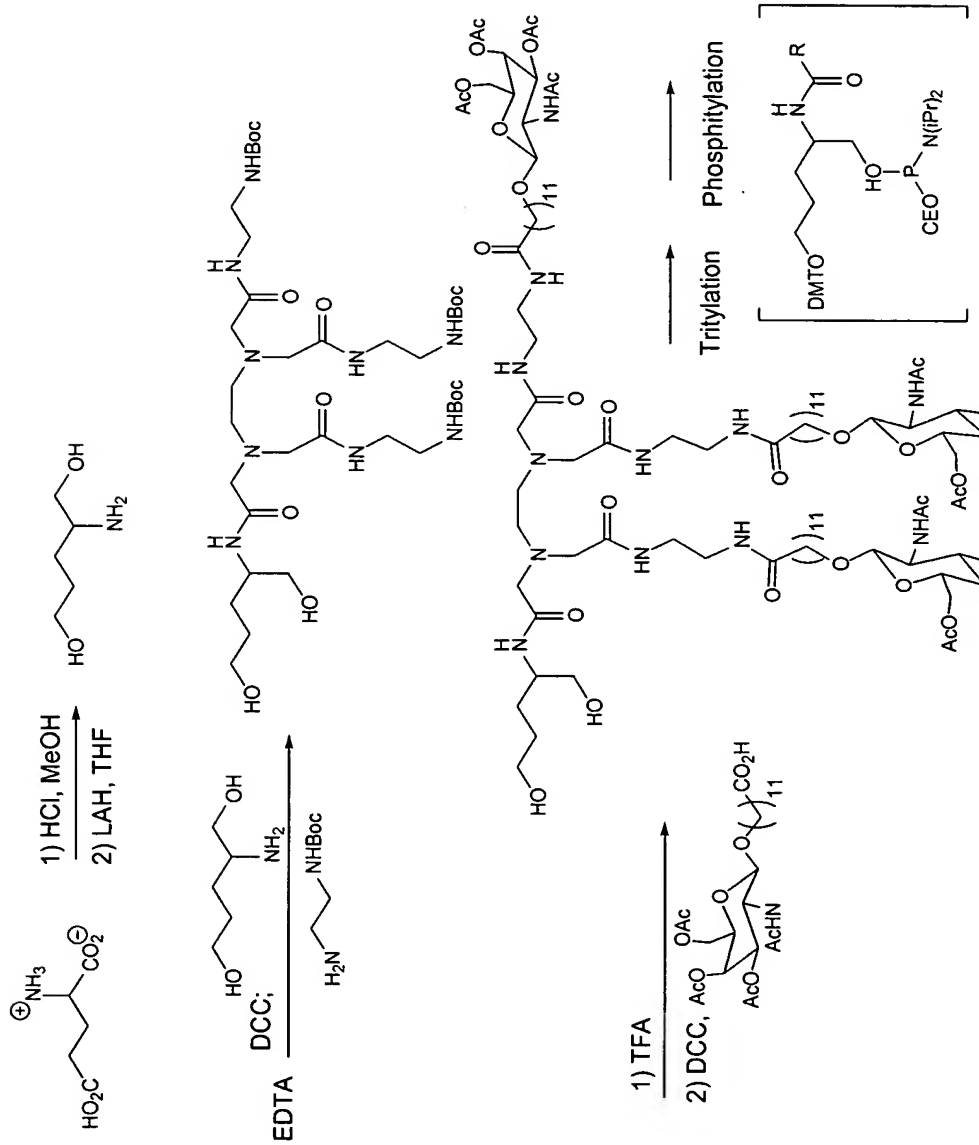


Figure 72: Alternate Synthesis of Tri-Galactosamine Conjugate

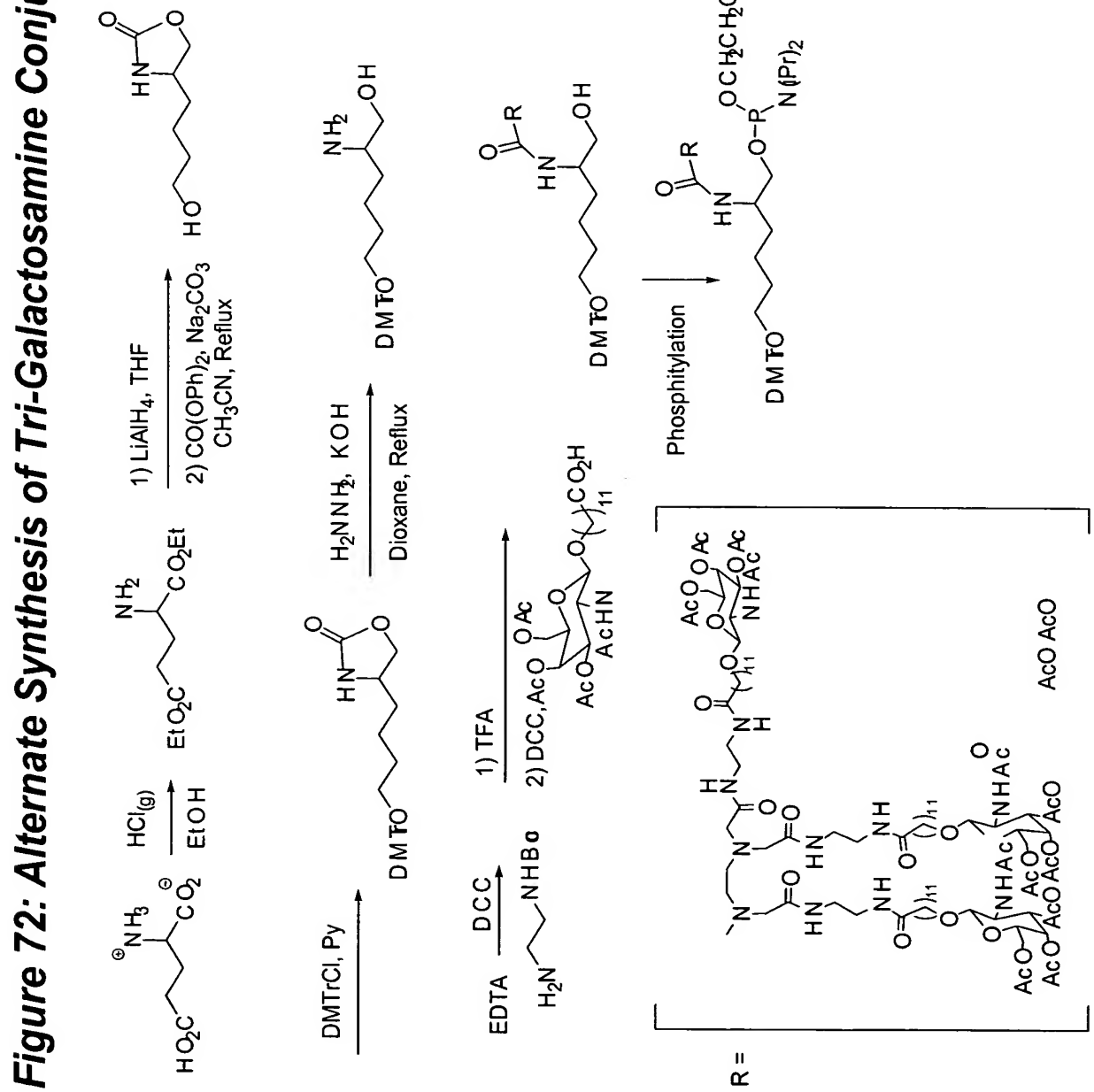


Figure 73: Synthesis of NHS Cholesterol Conjugate

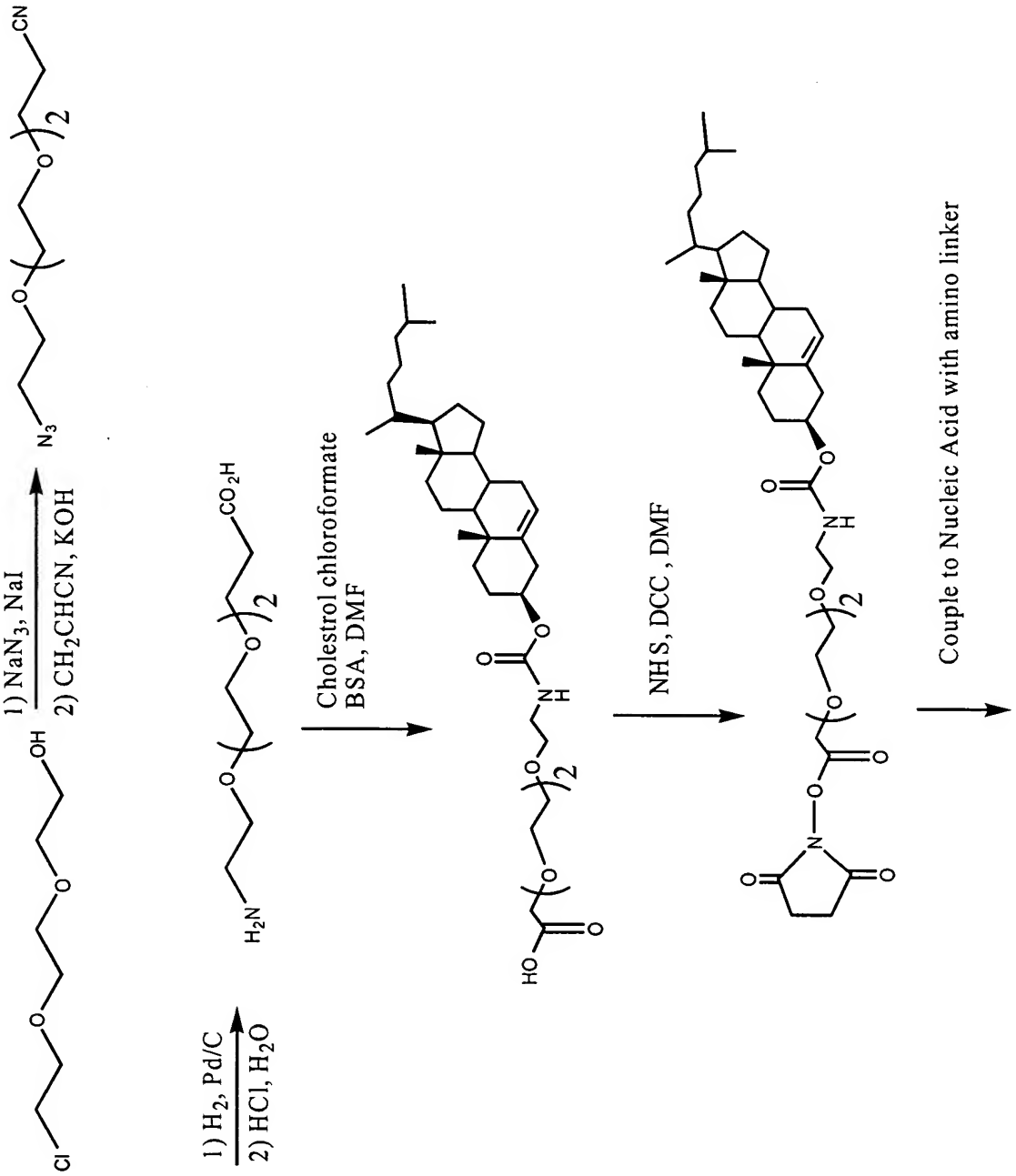
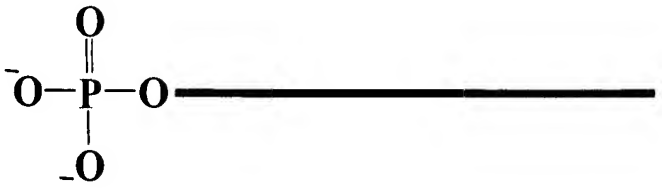
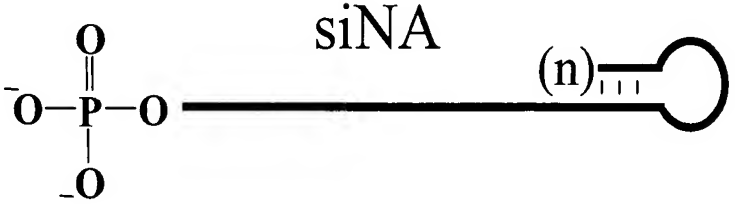


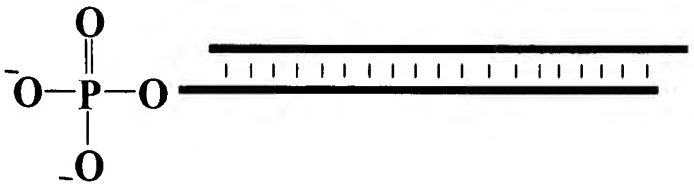
Figure 74: Phosphorylated siNA constructs



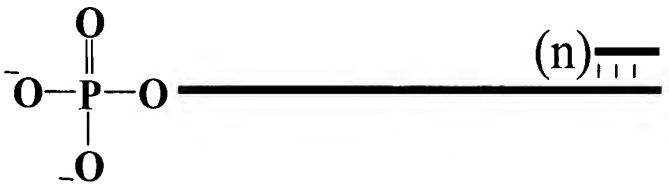
Phosphates can be modified as described herein

Asymmetric hairpin siNA





Asymmetric duplex siNA



(n) = number of base pairs (e.g. 3-18 bp)

Figure 75: 5'-phosphate modifications

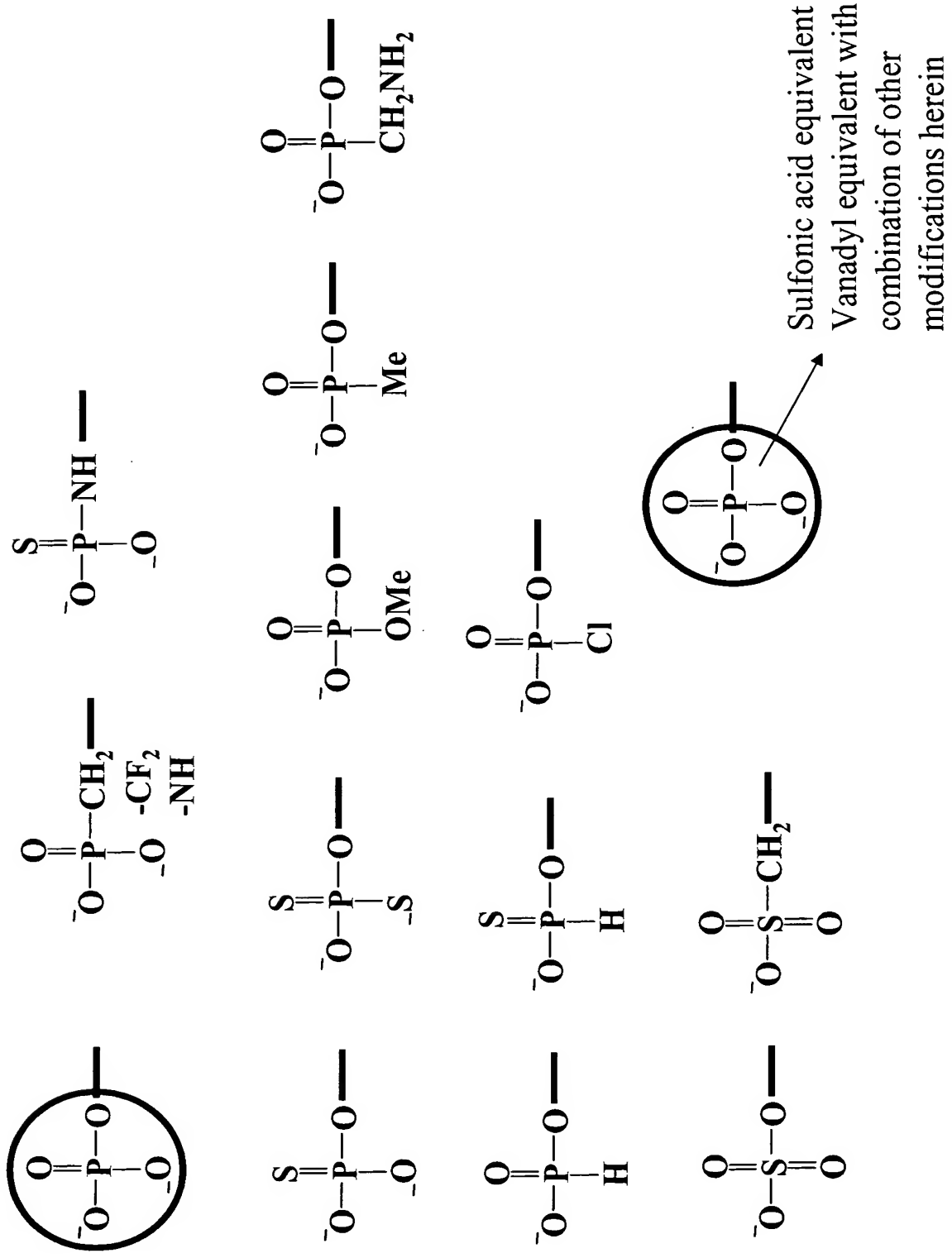


Figure 76: siNA Targeting VEGFR-1 Inhibits VEGF-Induced Rat Corneal Angiogenesis

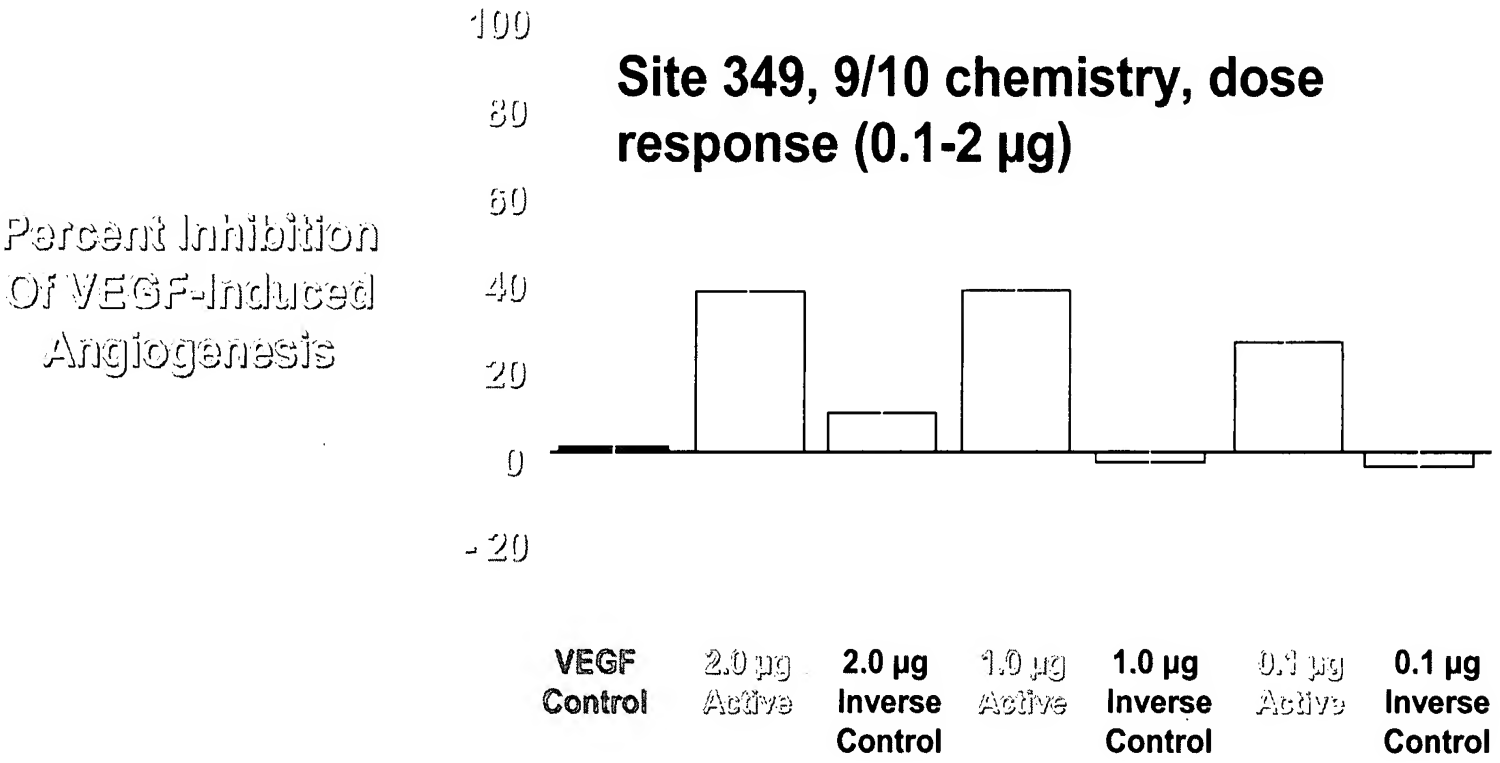


Figure 77: Duration of Effect of Modified siNA Constructs

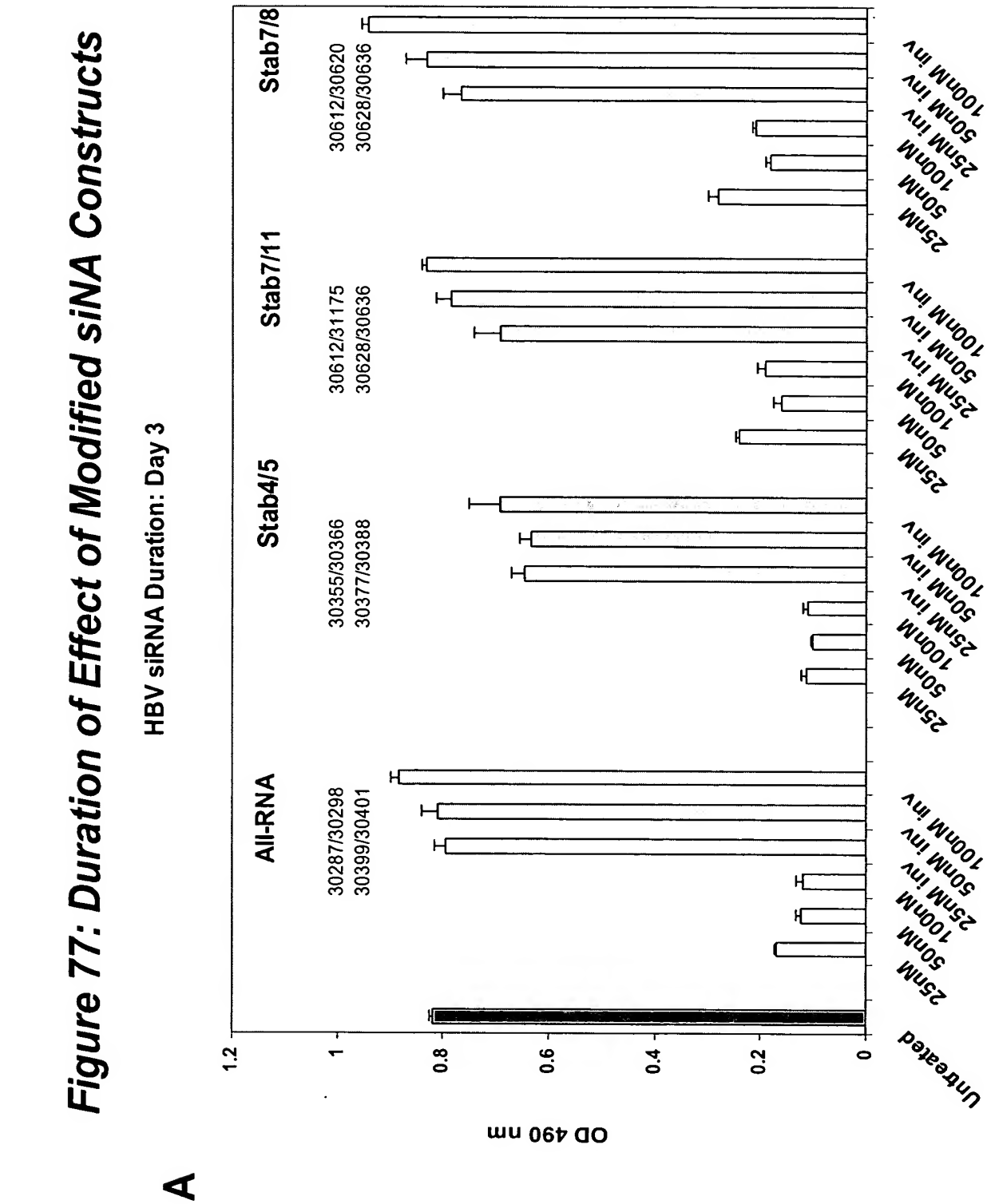
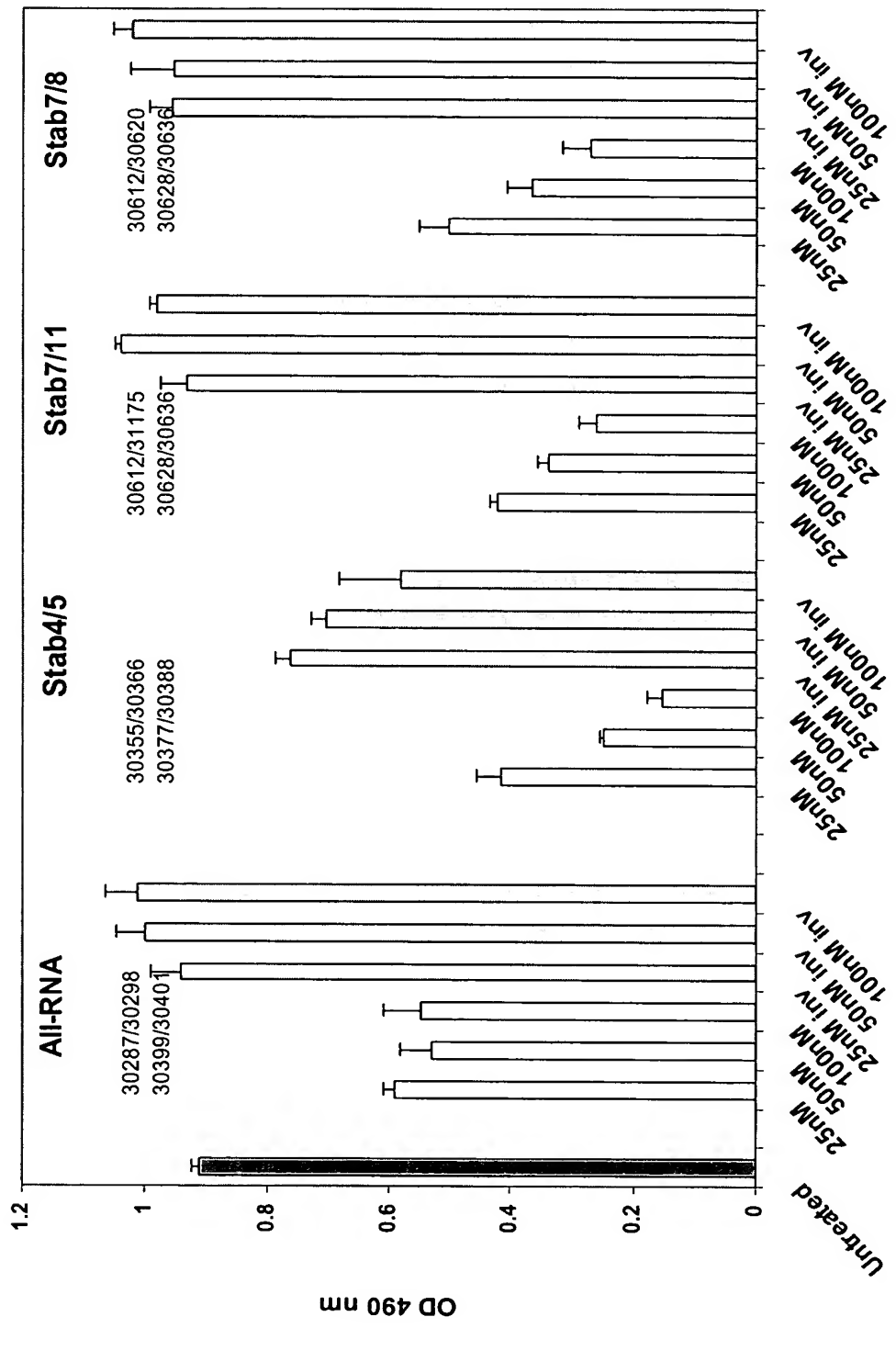


Figure 77: Duration of Effect of Modified siNA Constructs



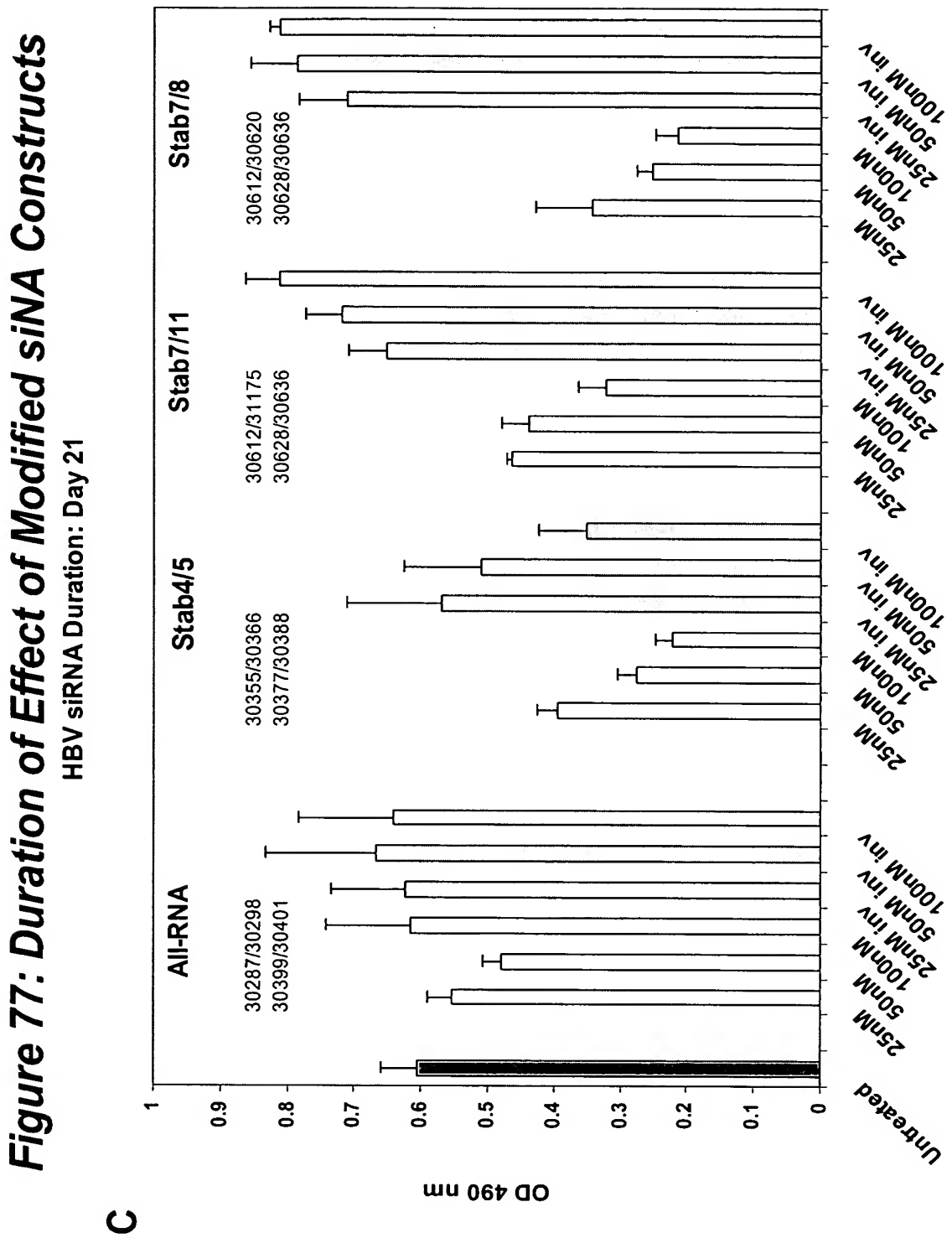
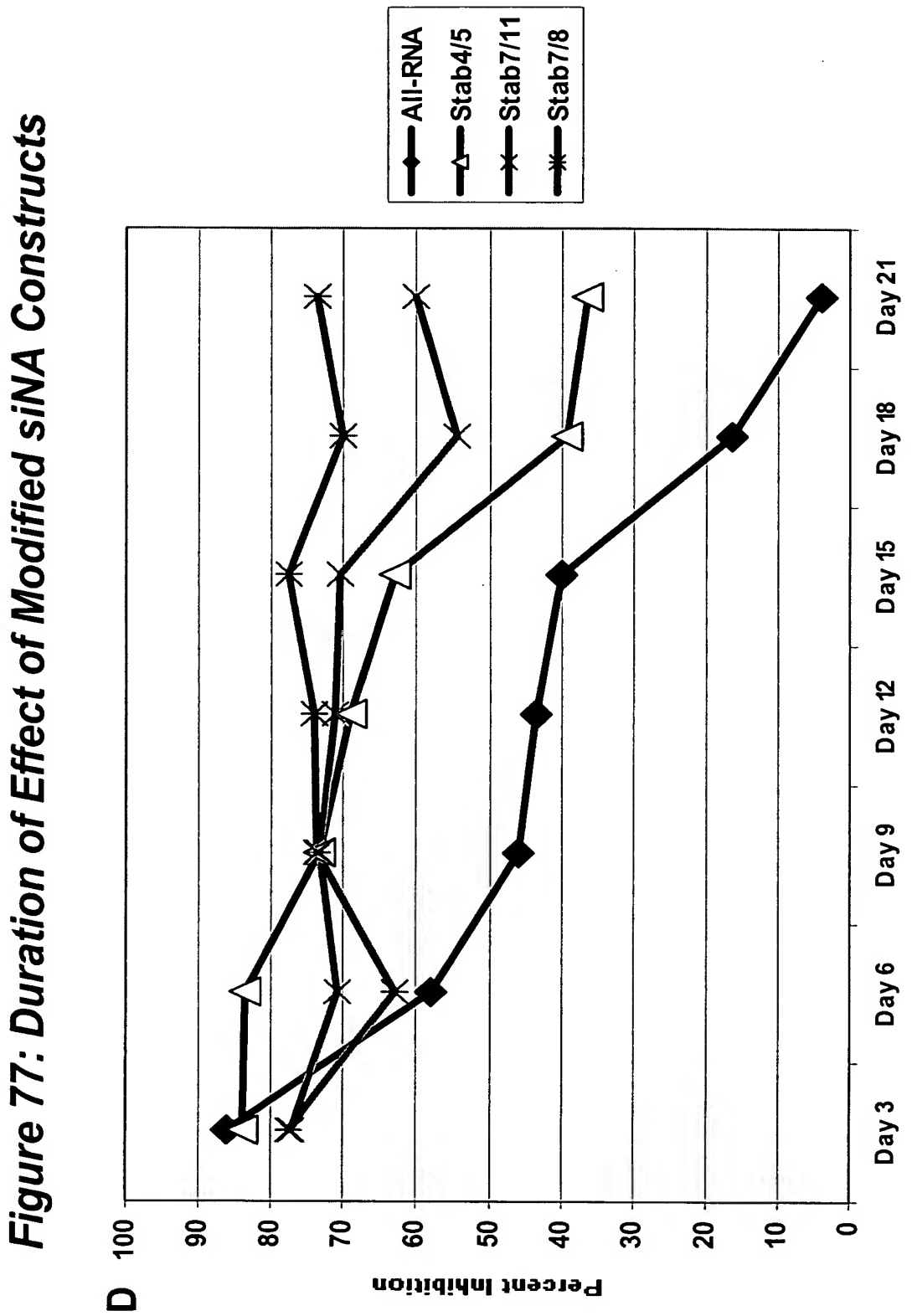
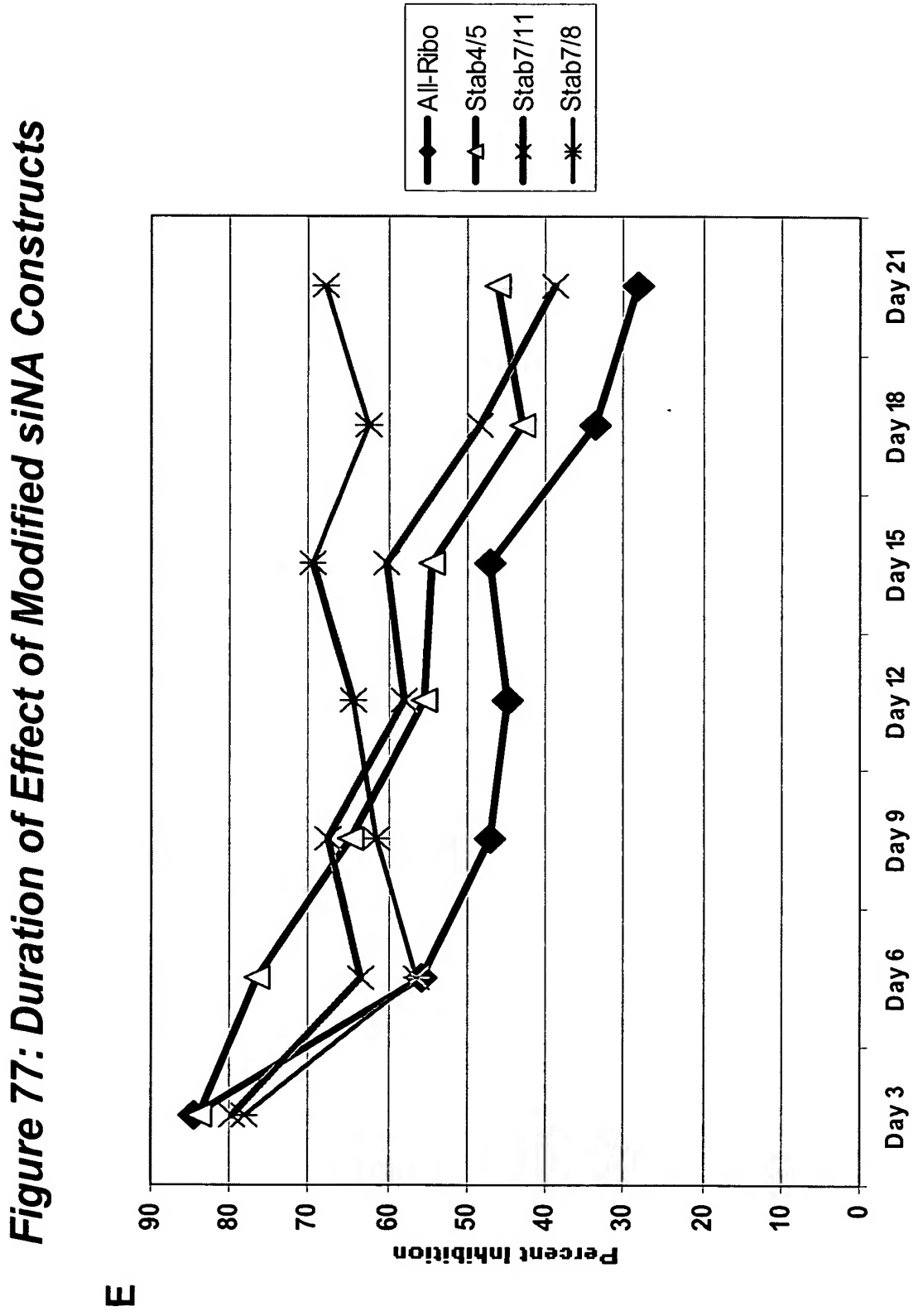


Figure 77: Duration of Effect of Modified siNA Constructs
HBV siRNA Duration: Day 21
C
OD 490 nm
1
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
All-RNA
30287/30298
30399/30401
Stab4/5
30355/30366
30377/30388
Stab7/11
30612/31175
30628/30636
Stab7/8
30612/30620
30628/30636
Untreated
25nM
50nM
100nM
25nM inv
50nM inv
100nM inv



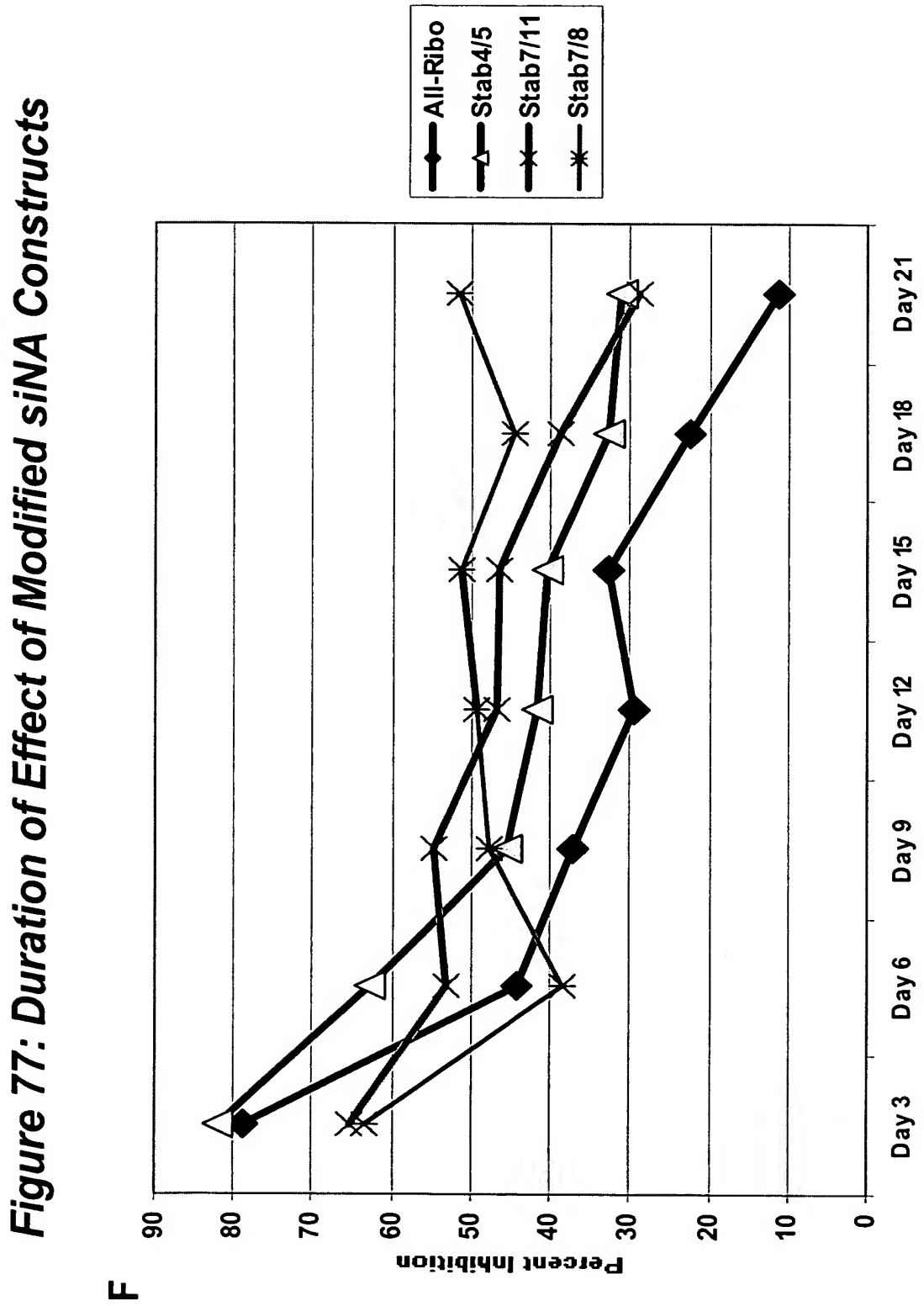
D

Figure 77: Duration of Effect of Modified siNA Constructs



E

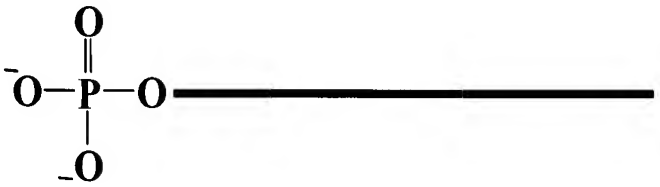
Figure 77: Duration of Effect of Modified siNA Constructs



F

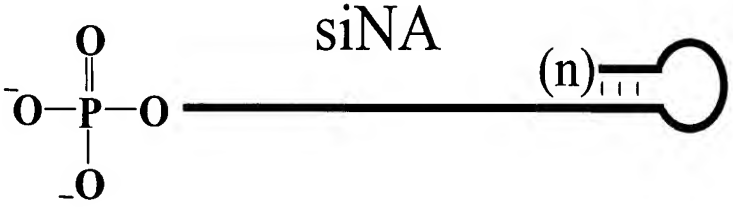
Figure 77: Duration of Effect of Modified siNA Constructs

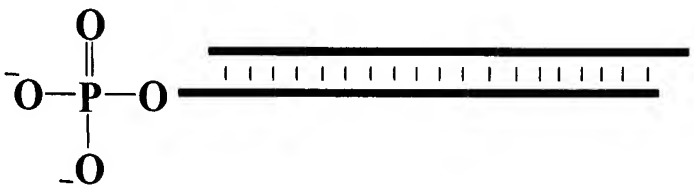
Figure 78: Phosphorylated siNA constructs



Phosphates can be modified as described herein

Asymmetric hairpin siNA





Asymmetric duplex siNA

(n) = number of base pairs (e.g. 3-18 bp)

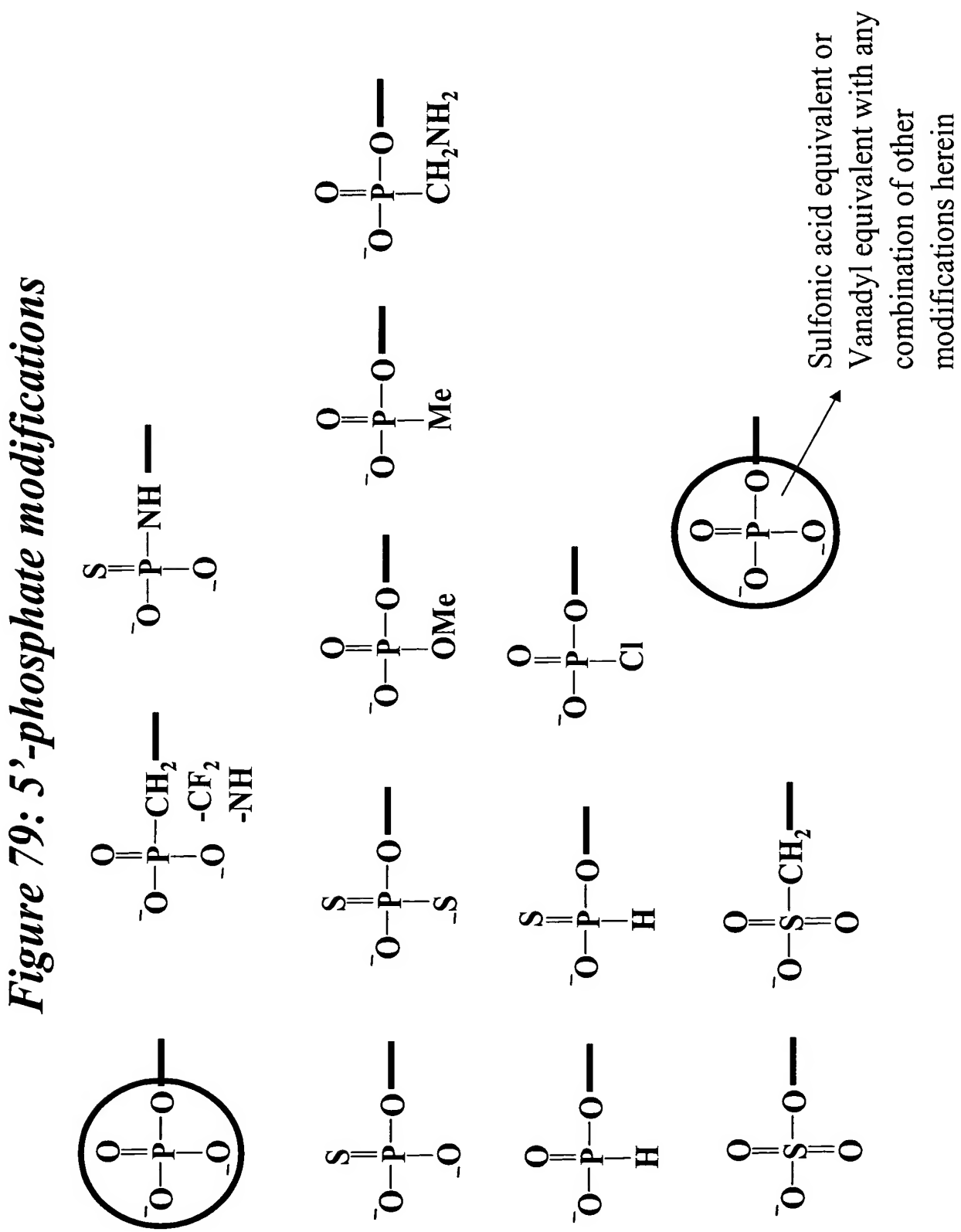


Figure 79: 5'-phosphate modifications
-NH
-CF2
Me
OMe
Cl
H
Sulfonic acid equivalent or
Vanadyl equivalent with any
combination of other
modifications herein

Figure 80: Serum HBV DNA in Mice Treated with siNA Via HDI

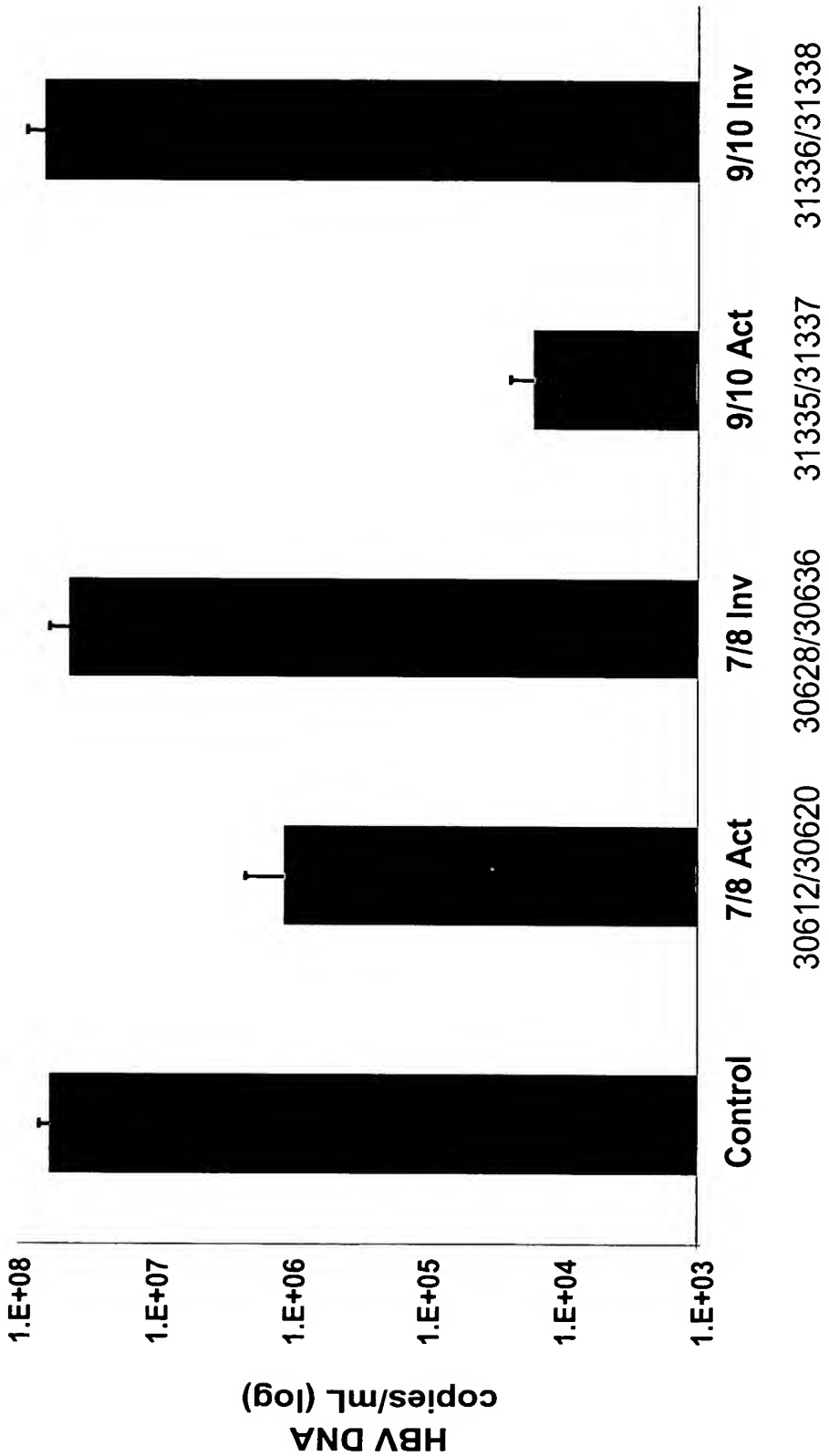
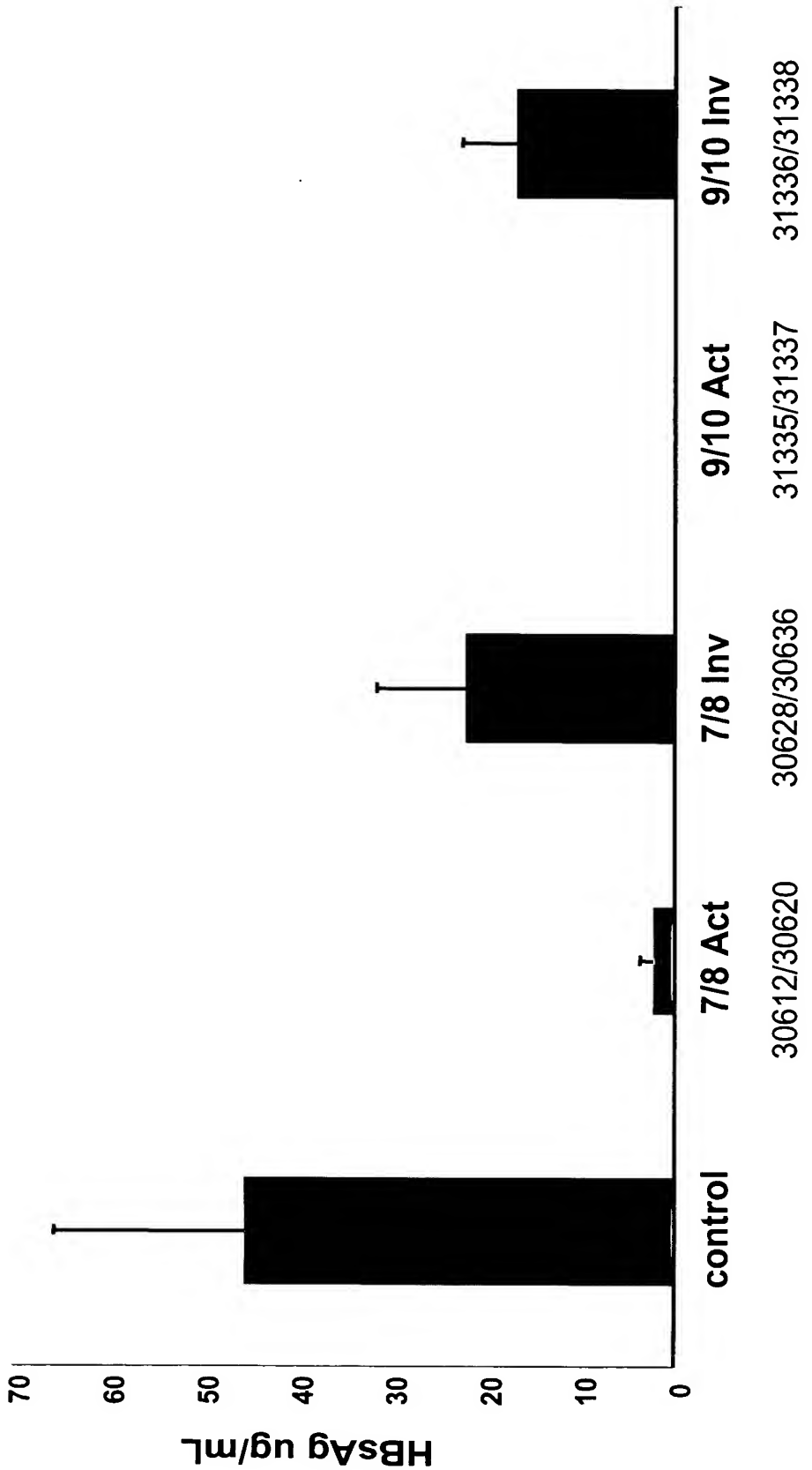


Figure 81: Serum HBsAg in Mice Treated with siNA Via HDI



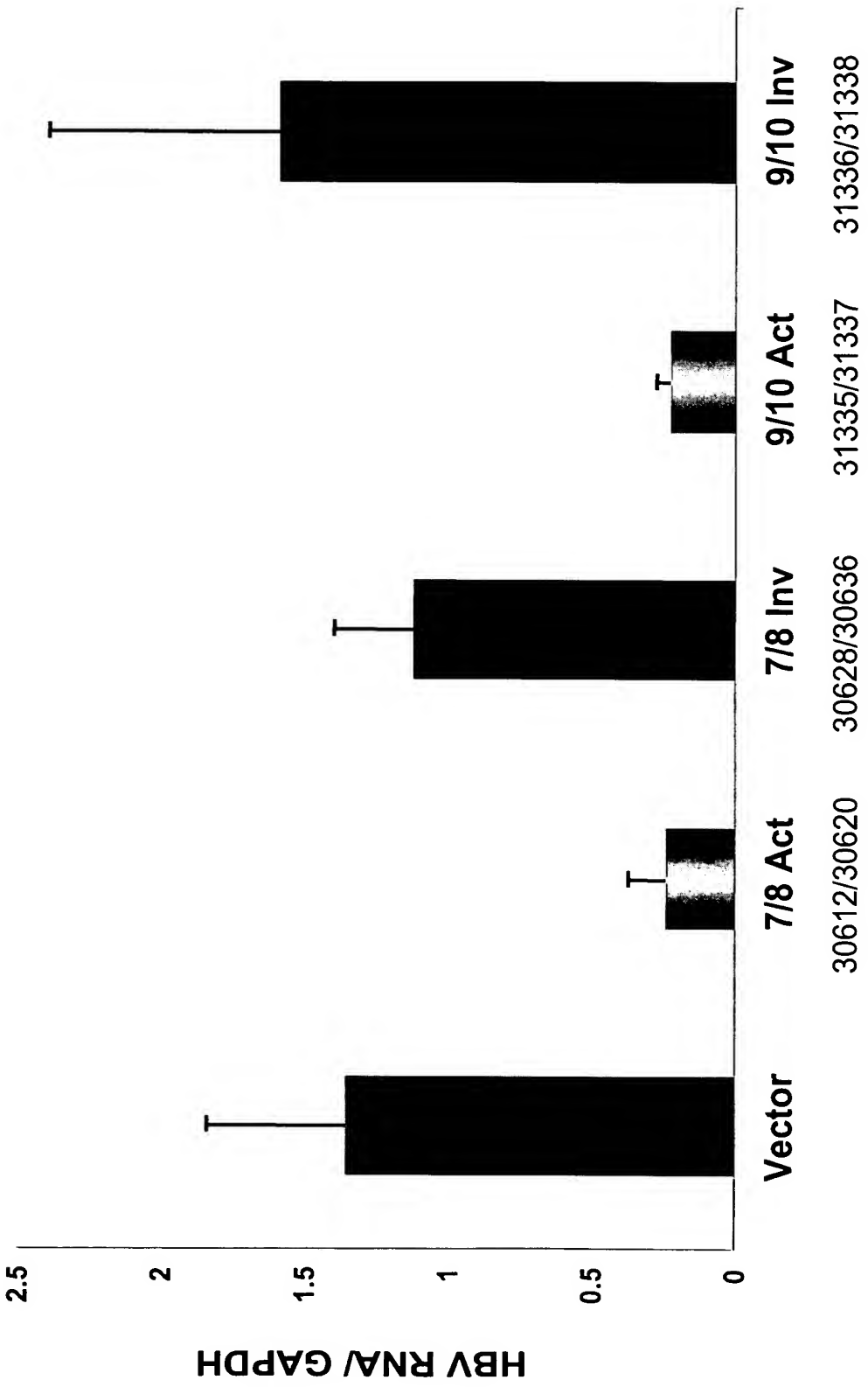


Figure 82: Liver HBV RNA in Mice Treated with siNA Via HDI

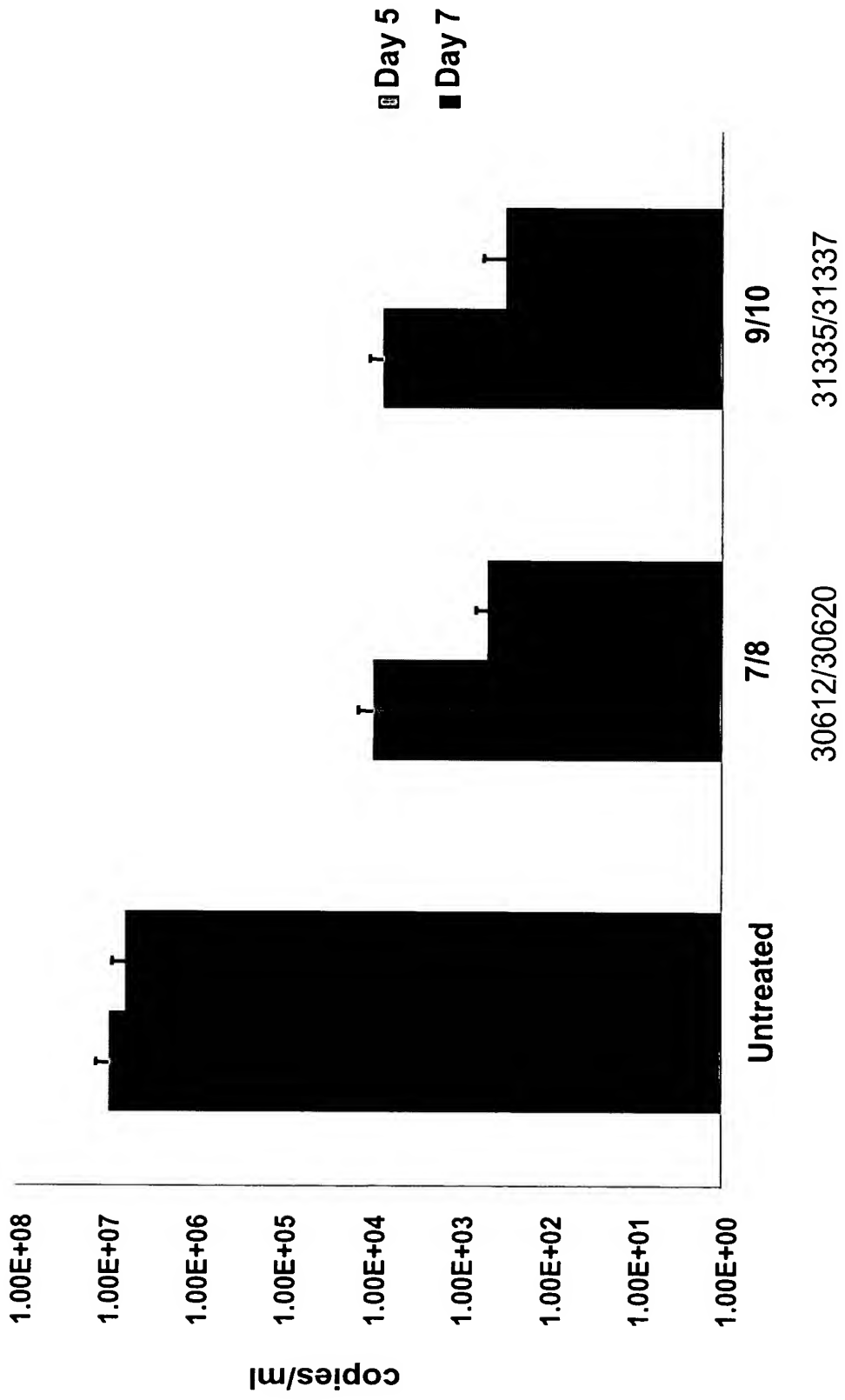


Figure 83: Serum HBV DNA in Mice Treated with siNA Via HDI 5 and 7 days post treatment

Figure 84: Luciferase Dose Response of select active siNA constructs

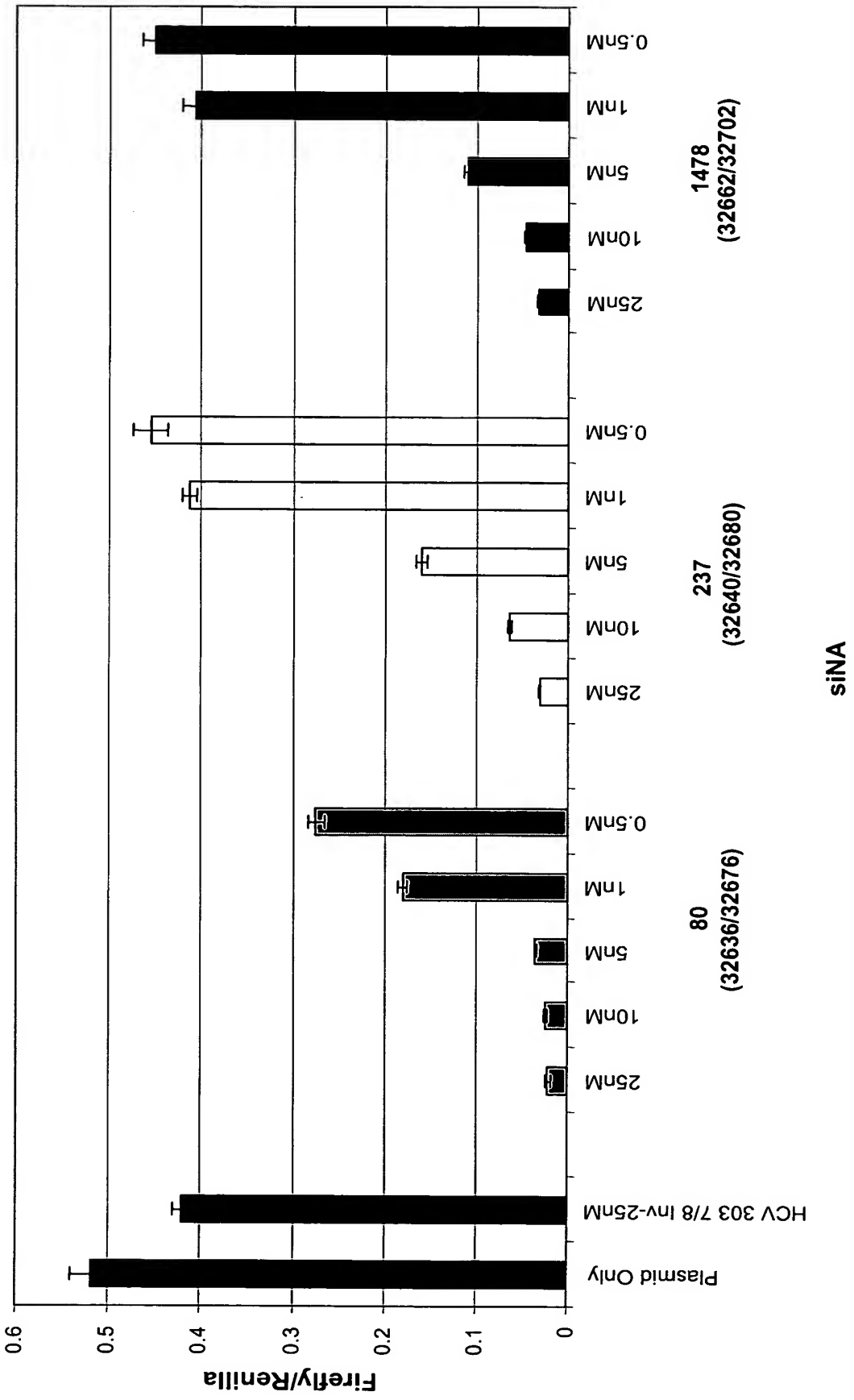
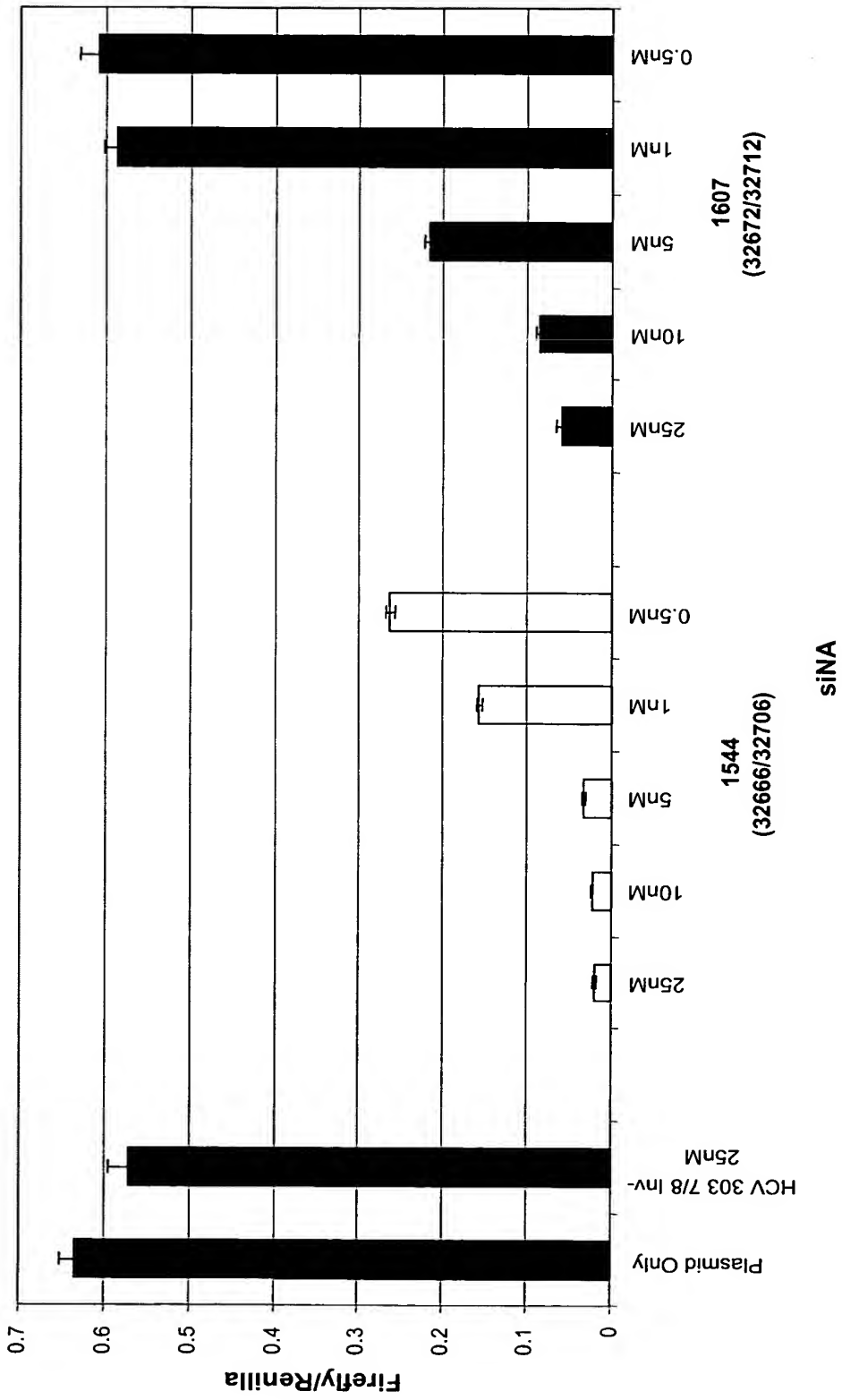


Figure 85: Luciferase Dose Response of select active siNA constructs



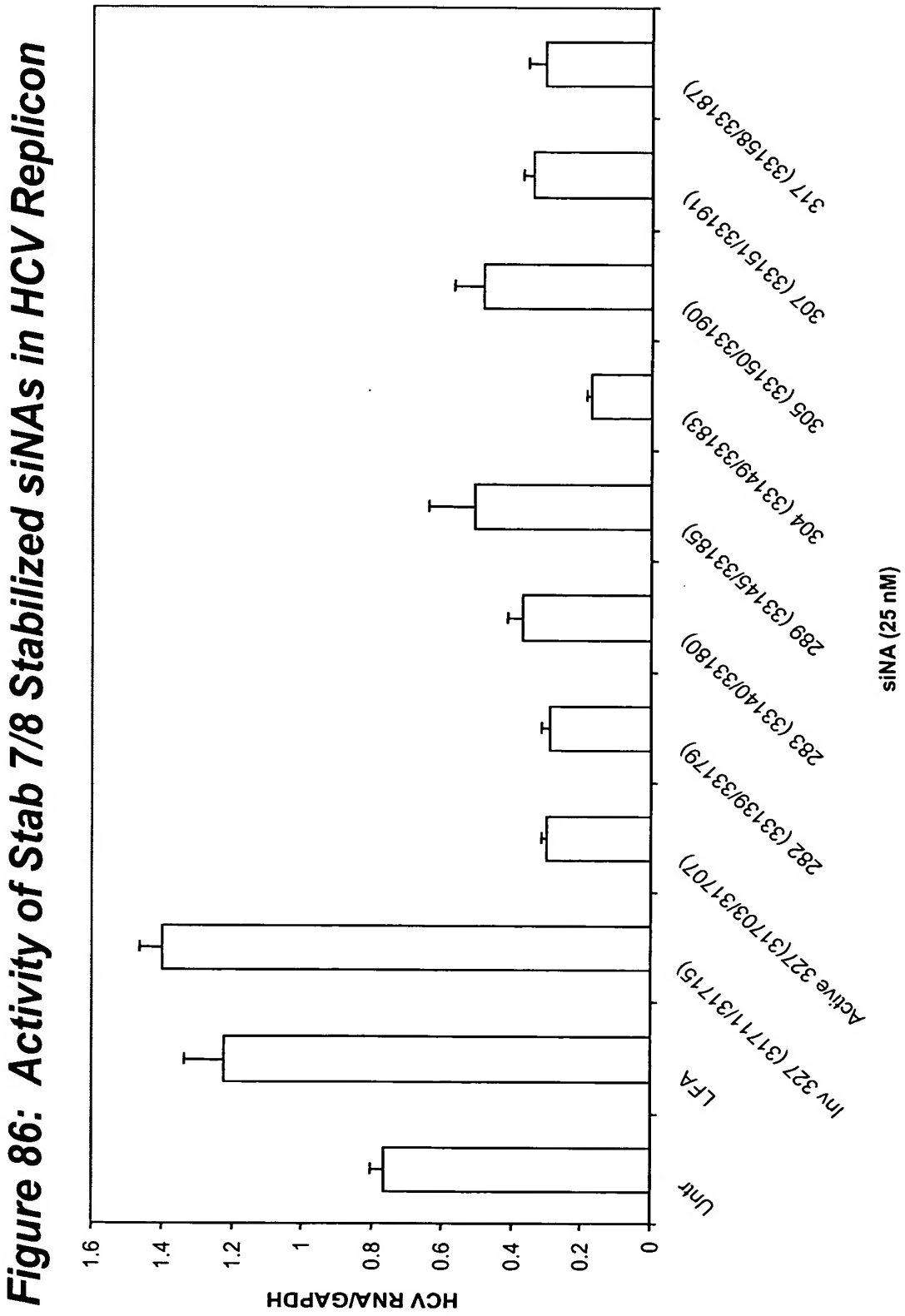


Figure 86: Activity of Stab 7/8 Stabilized siNAs in HCV Replicon
HCV RNA/GAPDH
0
0.2
0.4
0.6
0.8
1
1.2
1.4
1.6
siNA (25 nM)
Unitr
LFA
Inv 327 (31711/31715)
Active 327(31703/31707)
282 (31139/33179)
283 (31140/33180)
289 (31145/33185)
304 (31149/33183)
305 (31150/33190)
307 (31151/33191)
317 (31158/33187)

Figure 87: Activity of Stabilized 7/8 siNAs Against HBV in HepG2 Cells

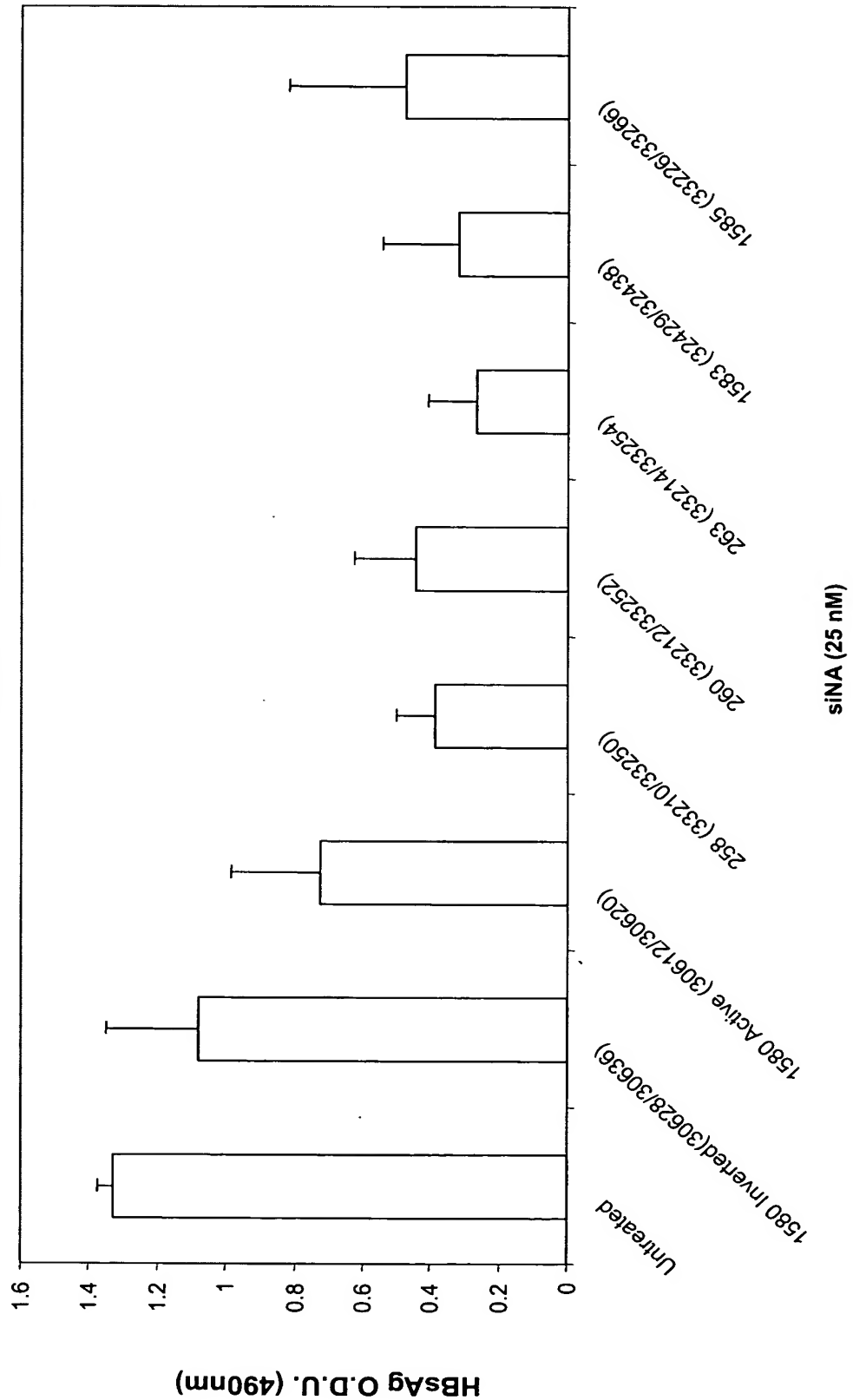


Figure 88: HBV/siNA to site 1580 Combination Constructs

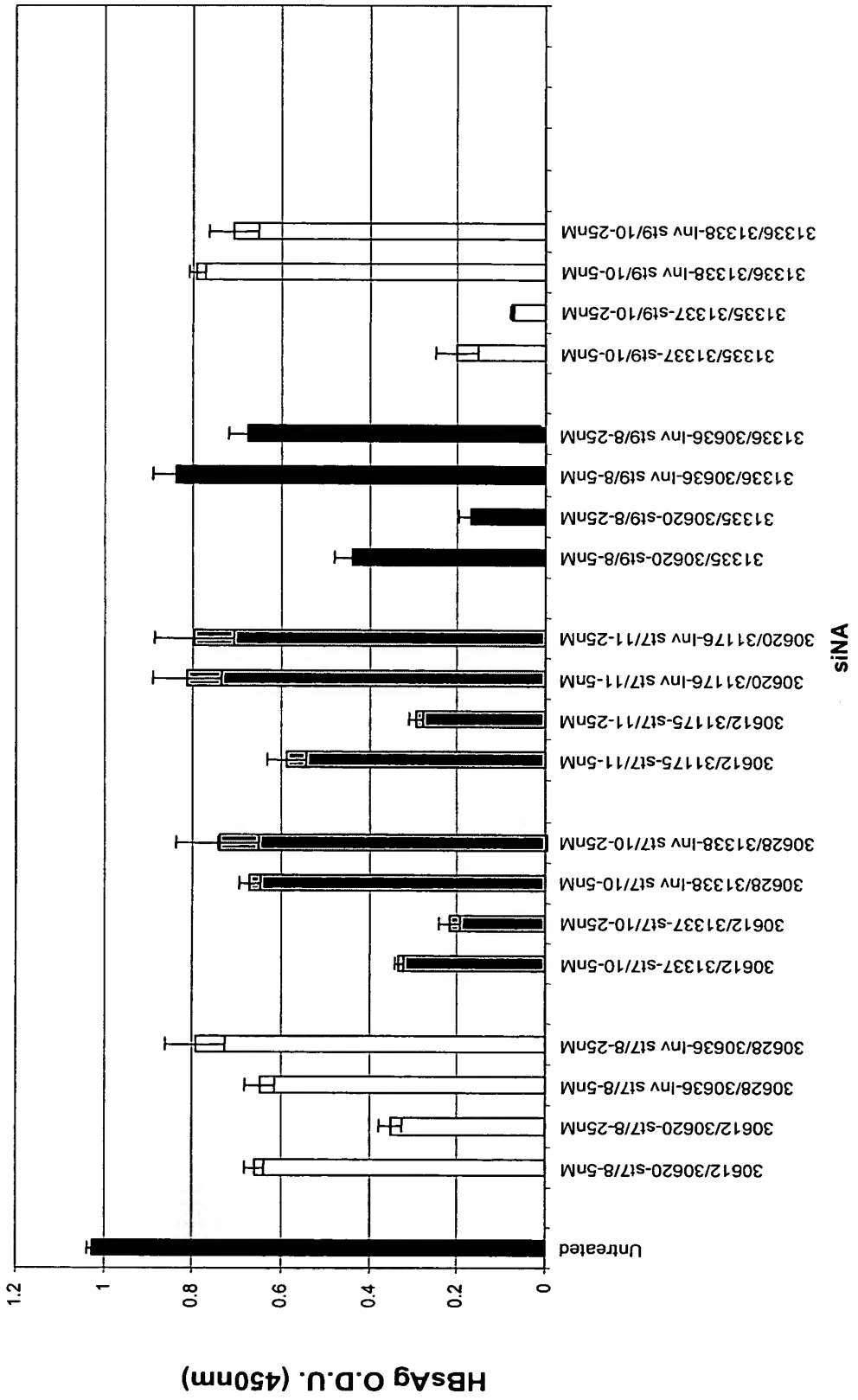
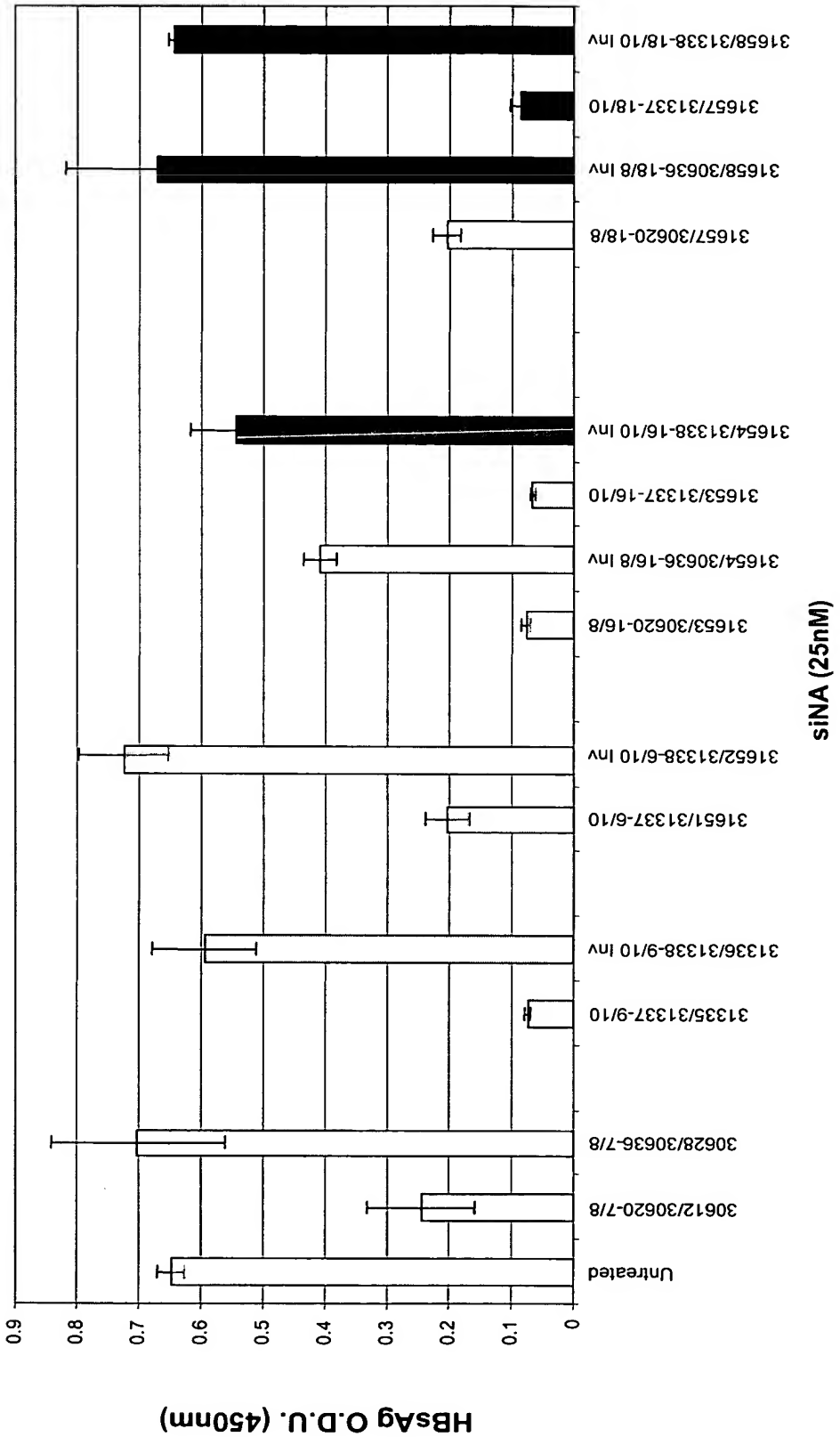


Figure 89: HBV/siNA to site 1580 Combination Constructs



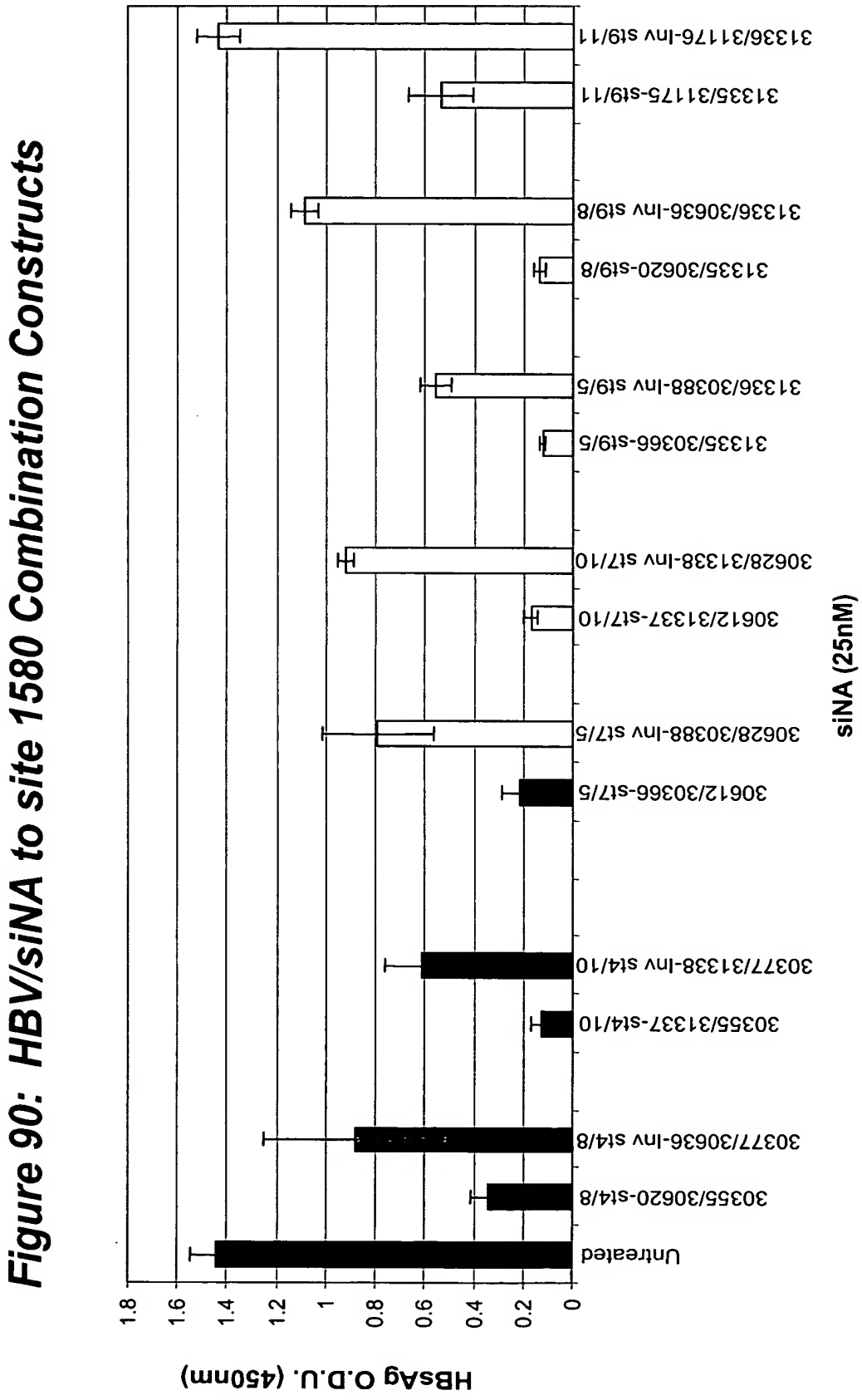
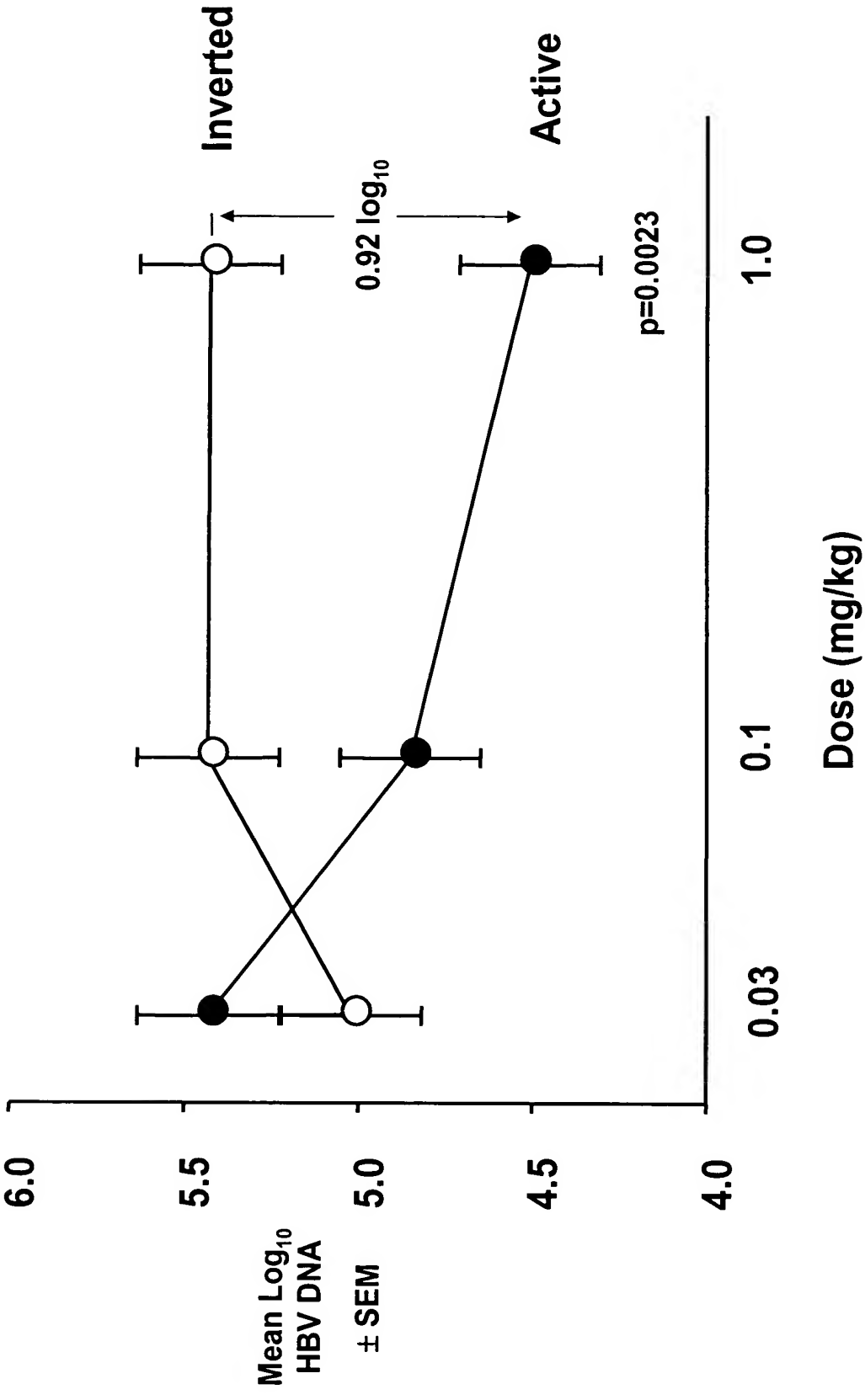


Figure 90: HBV/siNA to site 1580 Combination Constructs

Figure 91: PEI-Peg-Gal (3:1): Demonstration of Activity & Specificity HBV DNA



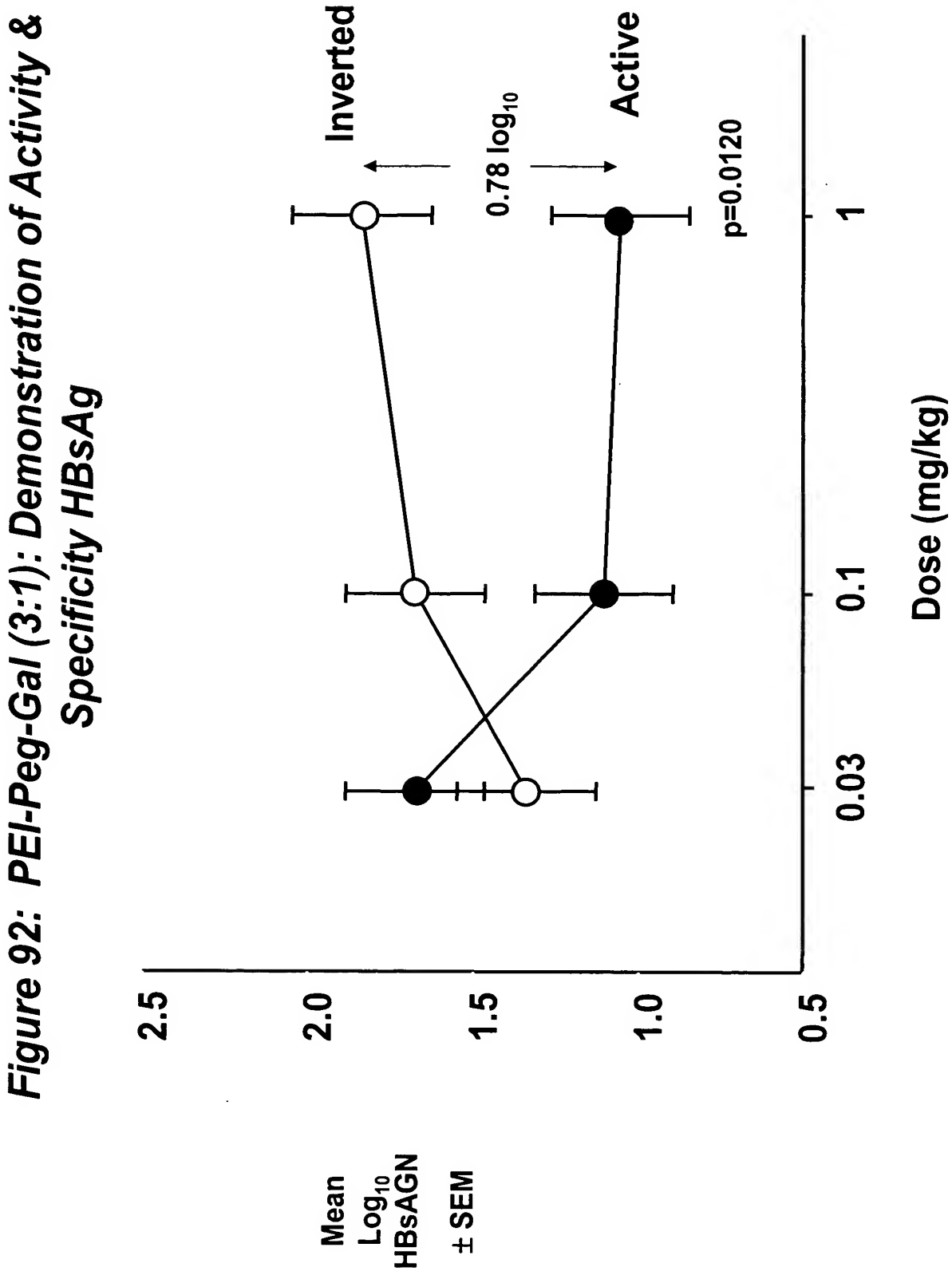


Figure 92: PEI-Peg-Gal (3:1): Demonstration of Activity & Specificity HBsAg

Figure 93: General Structure of PEI-PEG-TriGal with a lipid linker

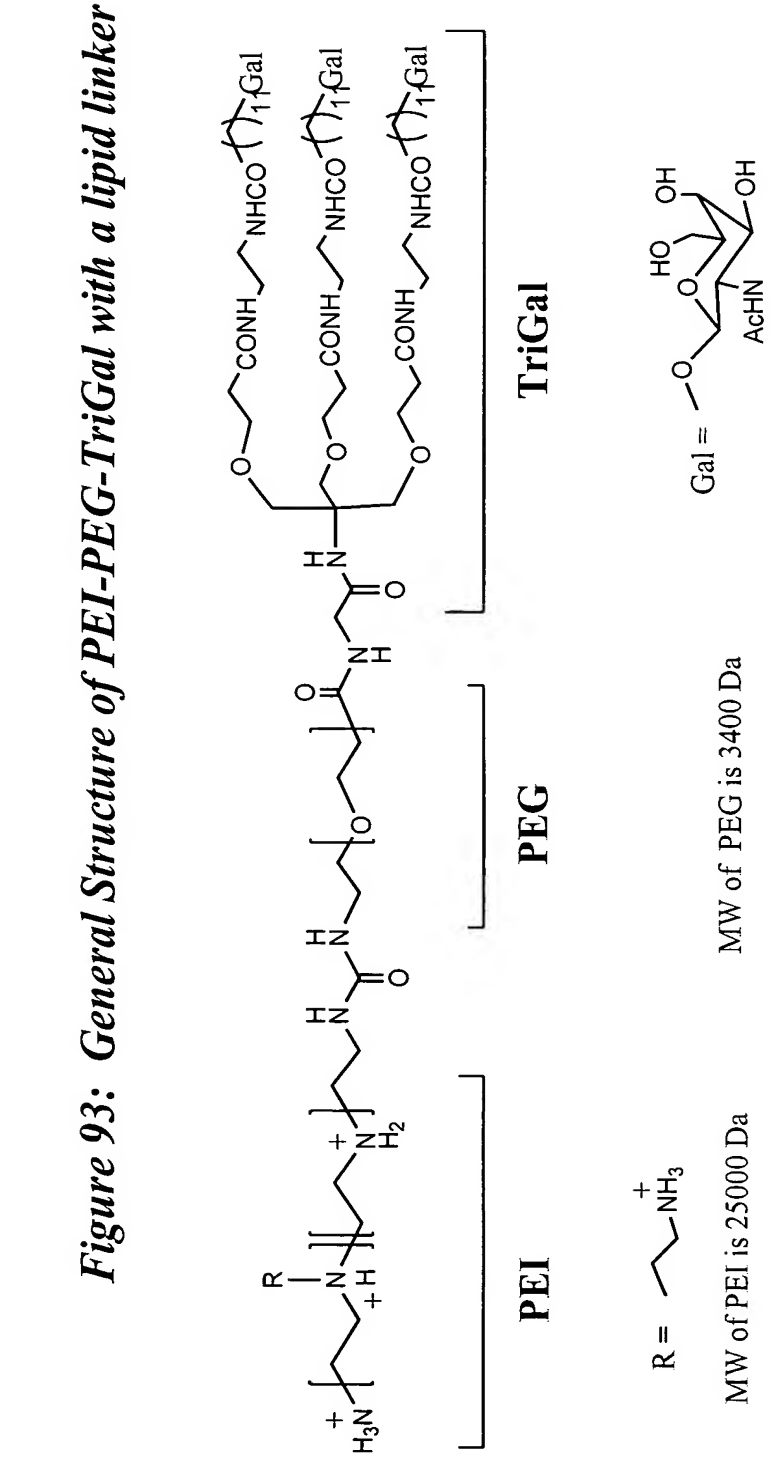


Figure 94A: Duplex forming oligonucleotide constructs that utilize palindrome or repeat sequences

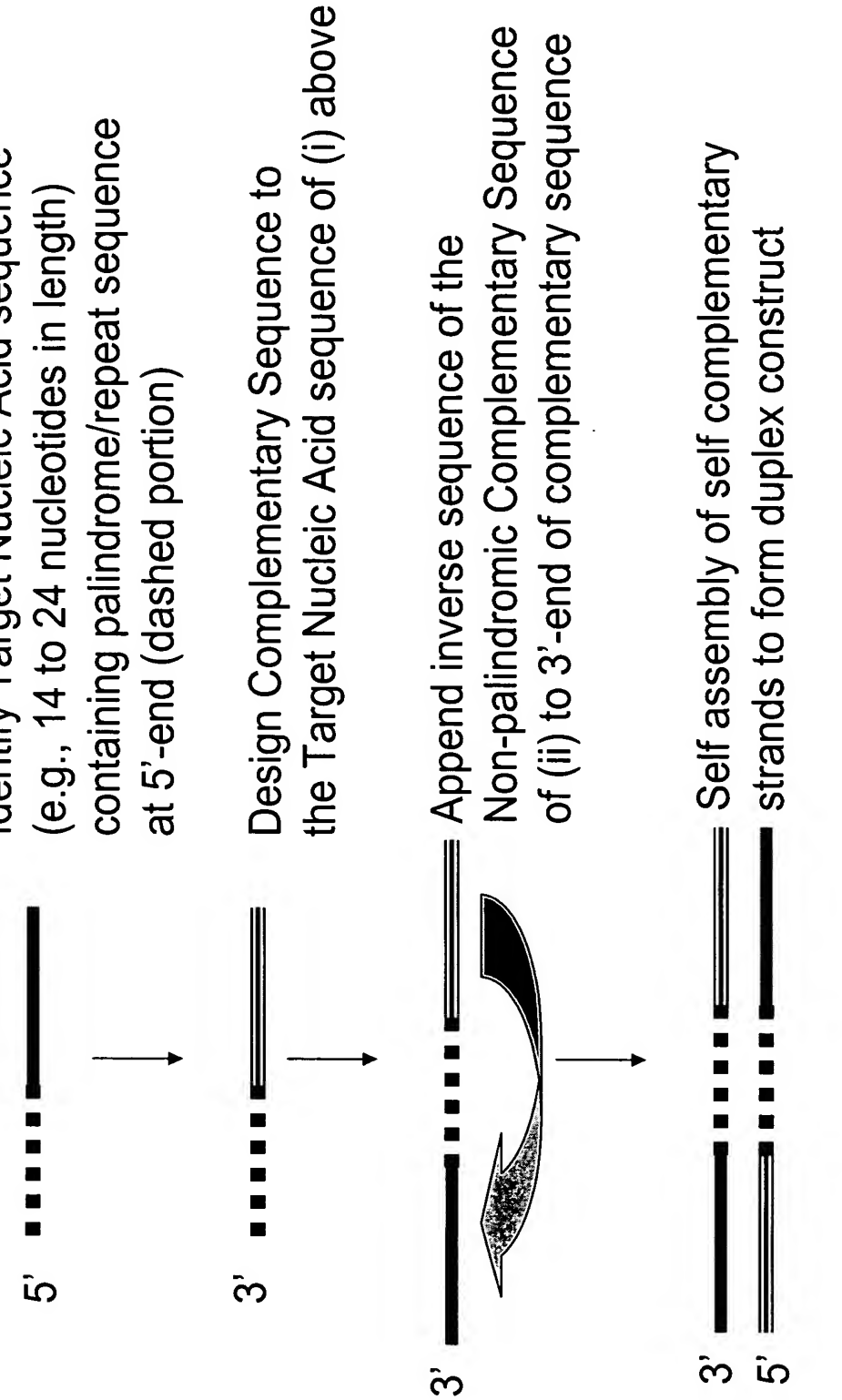
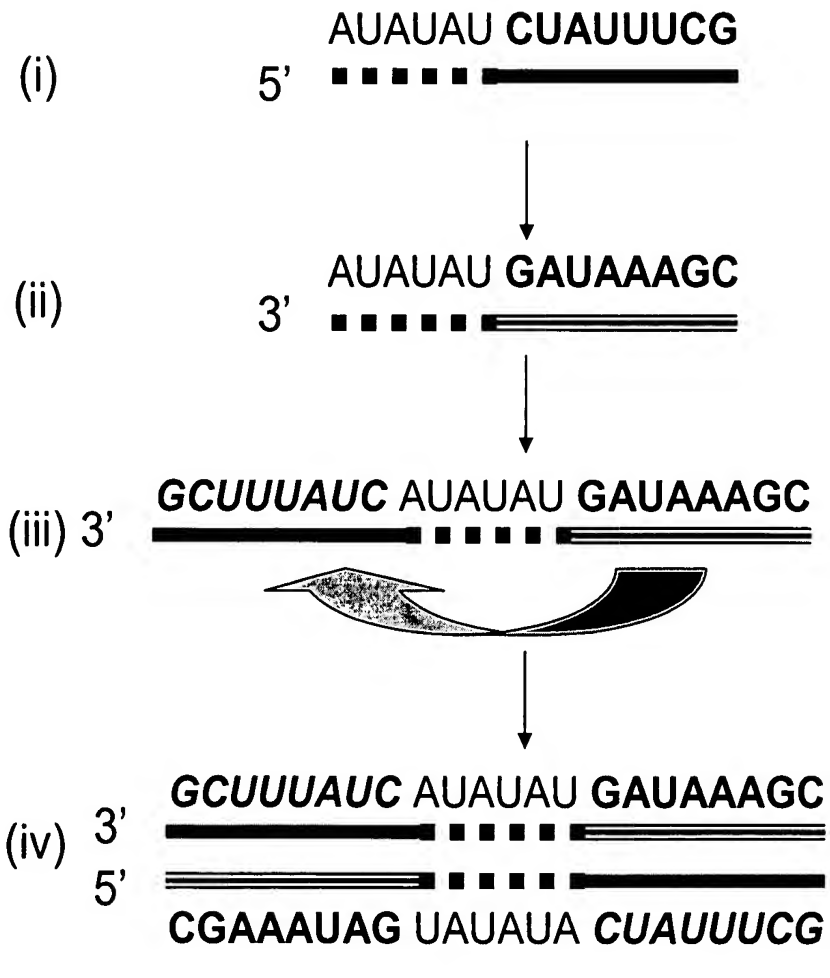


Figure 94B: Example of a duplex forming oligonucleotide sequence that utilizes a palindrome or repeat sequence



Identify Target Nucleic Acid sequence (e.g., 14 to 24 nucleotides in length) containing palindrome/repeat sequence at 5'-end (dashed portion)

Design Complementary Sequence to the Target Nucleic Acid sequence of (i) above

Append inverse sequence of the Non-palindromic Complementary Sequence of (ii) to 3'-end of complementary sequence

Self assembly of self complementary strands to form duplex construct (blunt ends)

Figure 94C: Example of a duplex forming oligonucleotide sequence that utilizes a palindrome or repeat sequence, self assembly

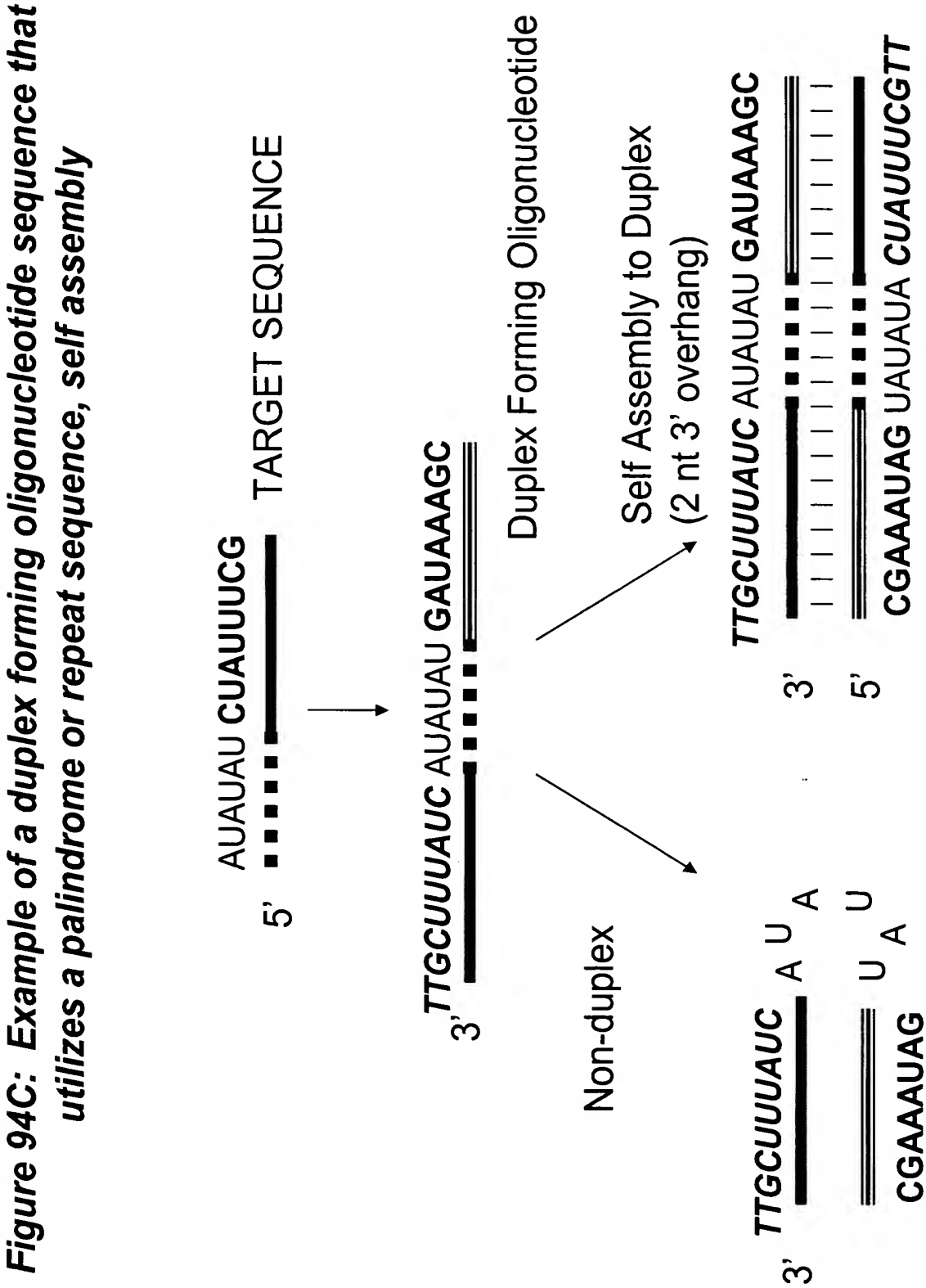


Figure 94D: Example of a duplex forming oligonucleotide sequence that utilizes a palindrome or repeat sequence, self assembly and inhibition of Target Sequence Expression

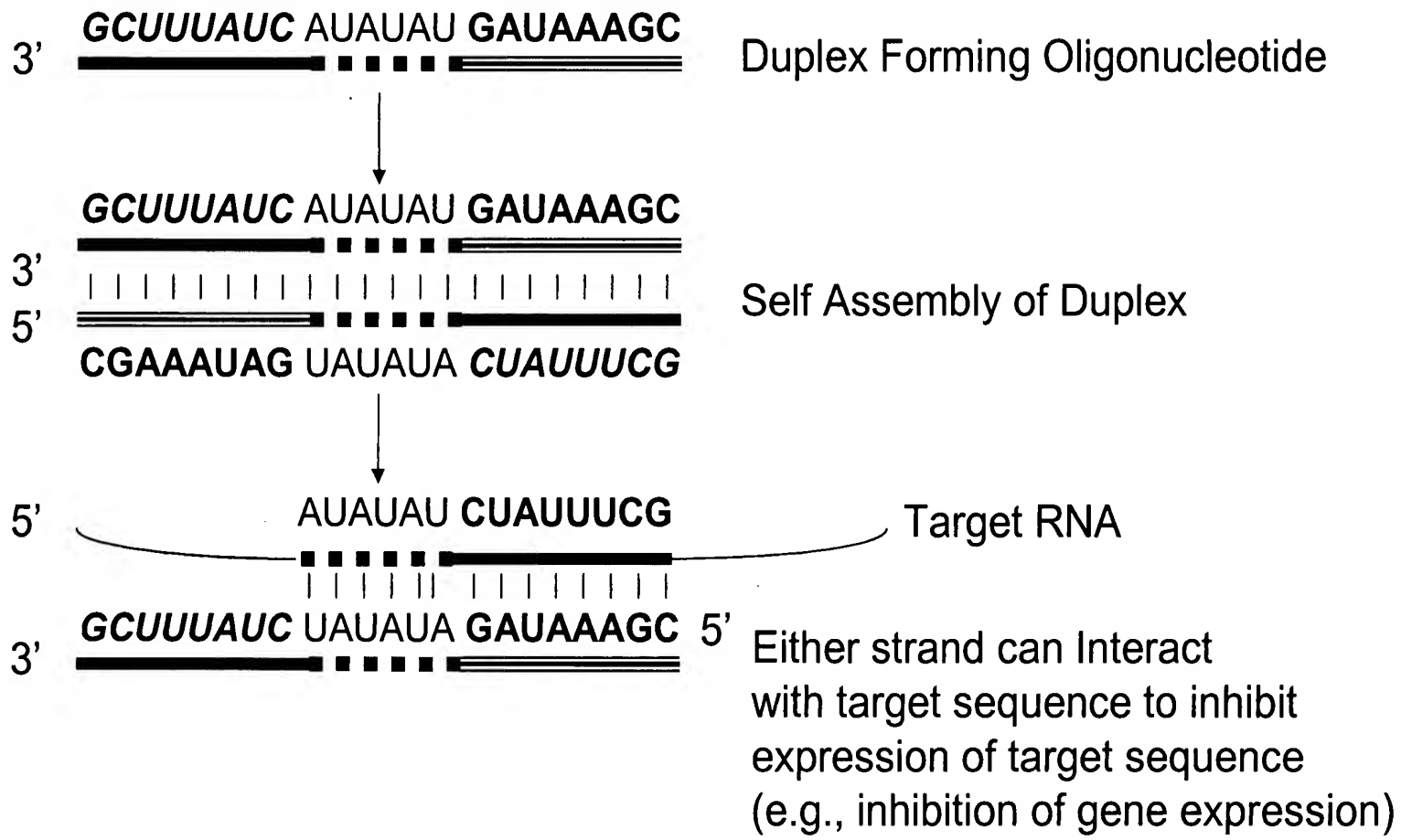
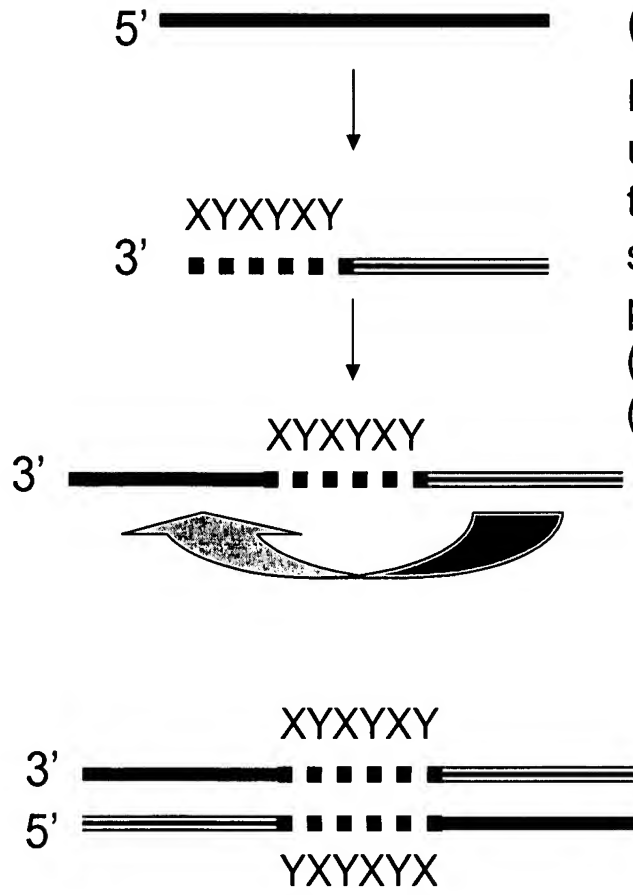


Figure 95: Duplex forming oligonucleotide constructs that utilize artificial palindrome or repeat sequences



Identify Target Nucleic Acid sequence (e.g., 14 to 24 nucleotides in length)

Design Complementary Sequence and utilize modified nucleotides (shown as X, Y) that interact with a portion of the target sequence and result in the formation of a palindrome/repeat sequence (e.g., 2 to 12 nucleotides) at 3'-end (dashed portion)

Append inverse sequence of Complementary region to 3'-end of palindrome/repeat sequence

Hybridize self complementary strands to form duplex siNA construct

Figure 96: Examples of double stranded multifunctional siNA constructs with distinct complementary regions

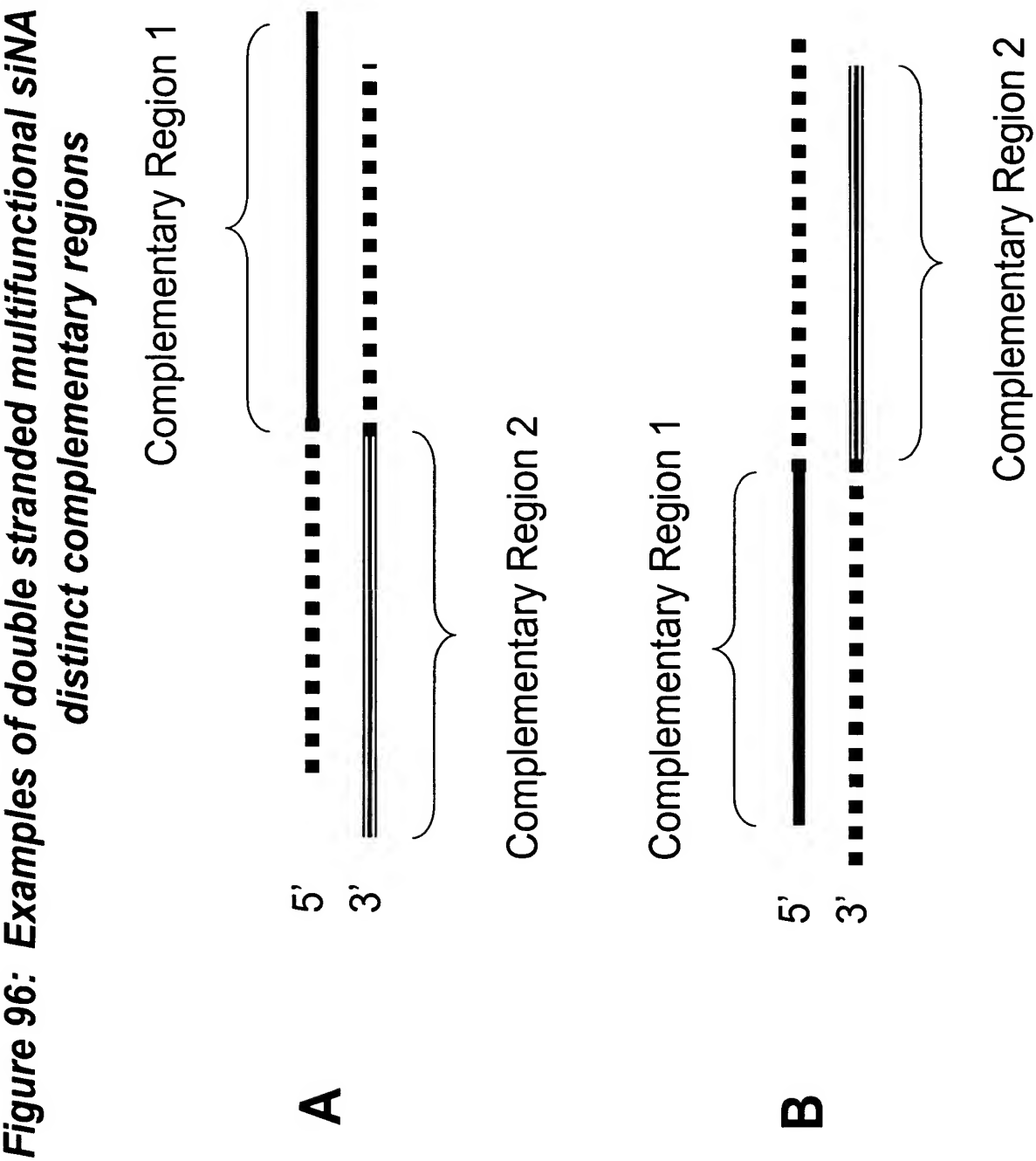


Figure 97: Examples of hairpin multifunctional siNA constructs with distinct complementary regions

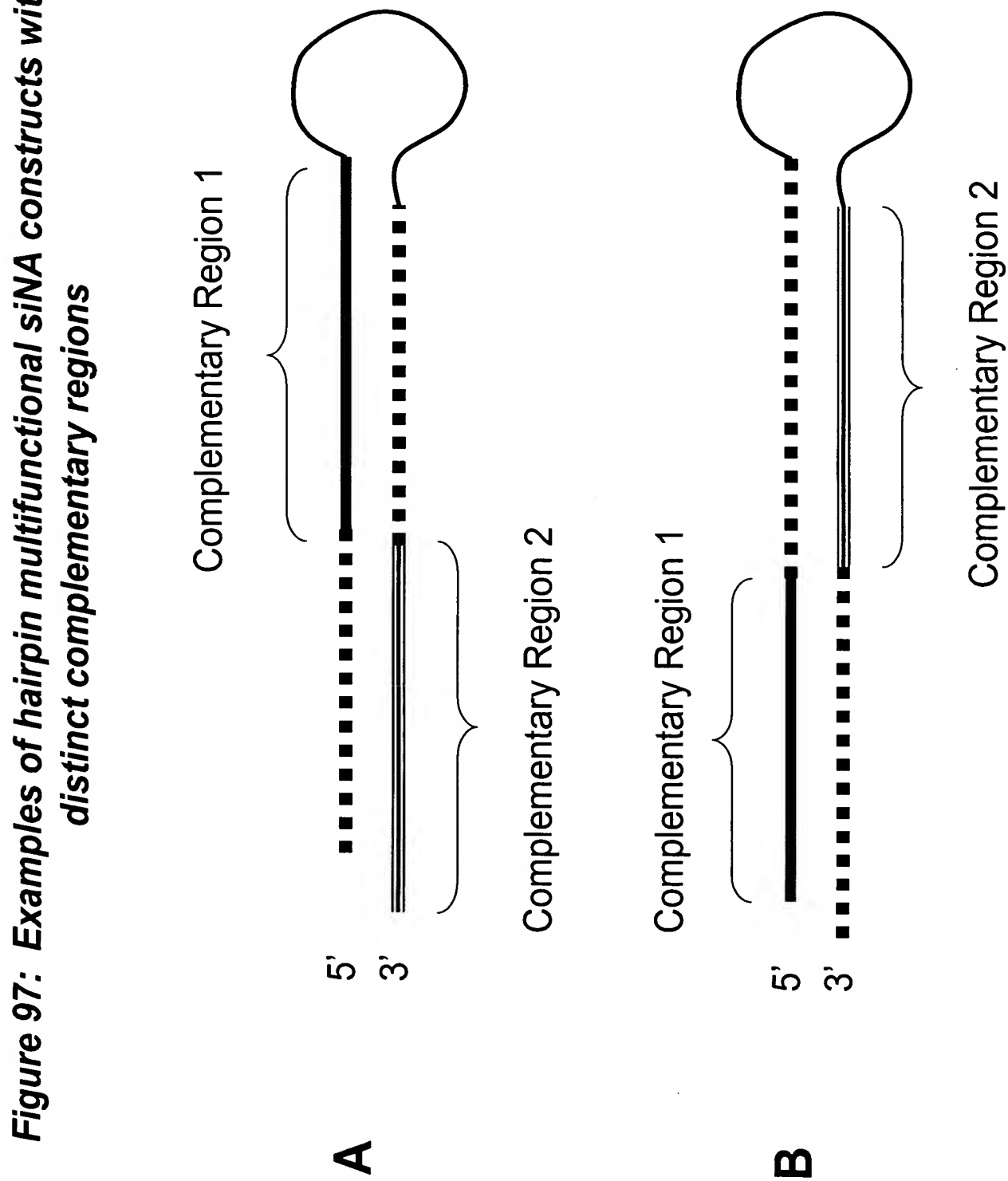


Figure 98: Examples of double stranded multifunctional siNA constructs with distinct complementary regions and a self complementary/palindrome region

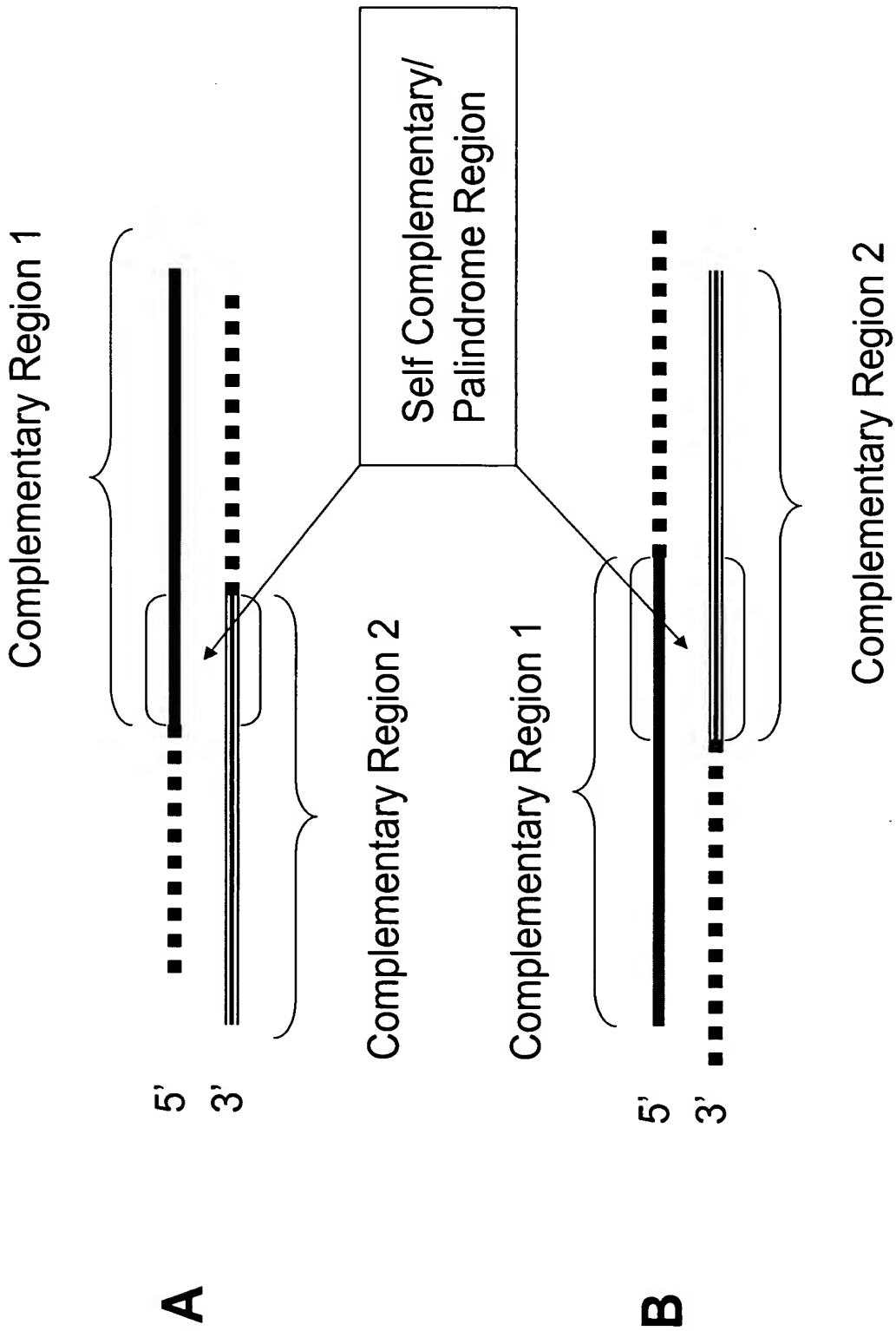
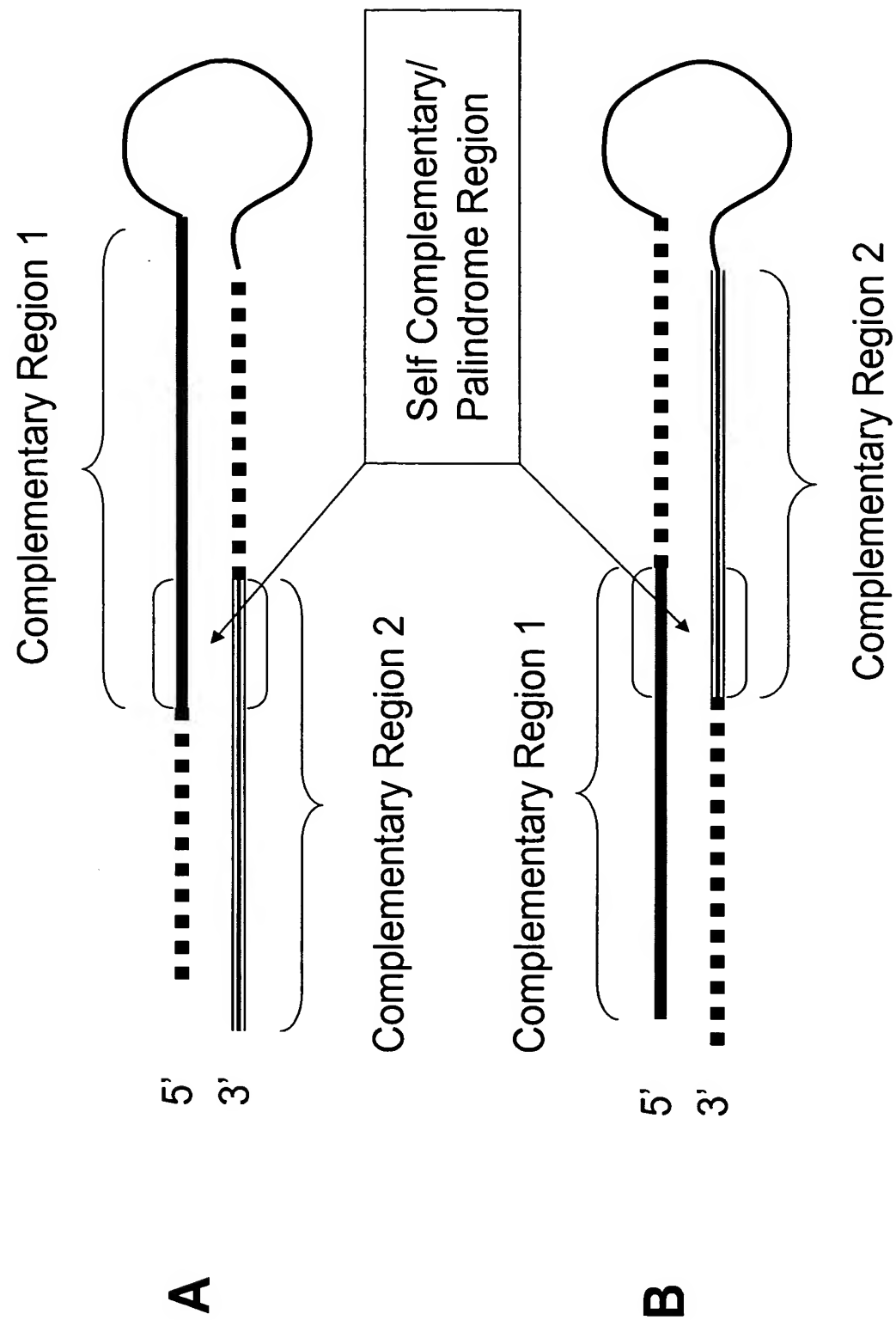


Figure 99: Examples of hairpin multifunctional siNA constructs with distinct complementary regions and a self complementary/palindrome region



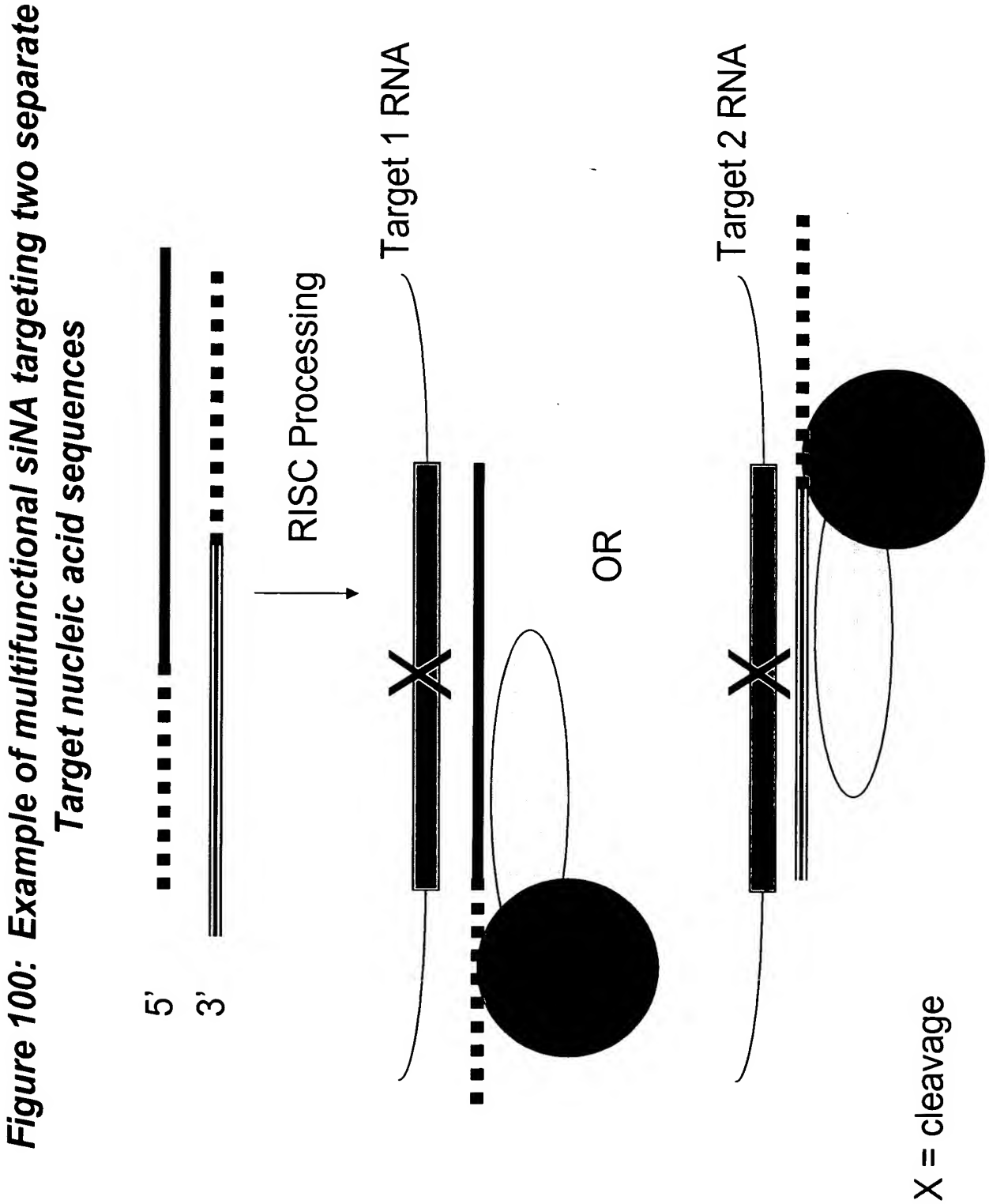


Figure 100: Example of multifunctional siNA targeting two separate Target nucleic acid sequences

Figure 101: Example of multifunctional siNA targeting two regions within the same target nucleic acid sequence

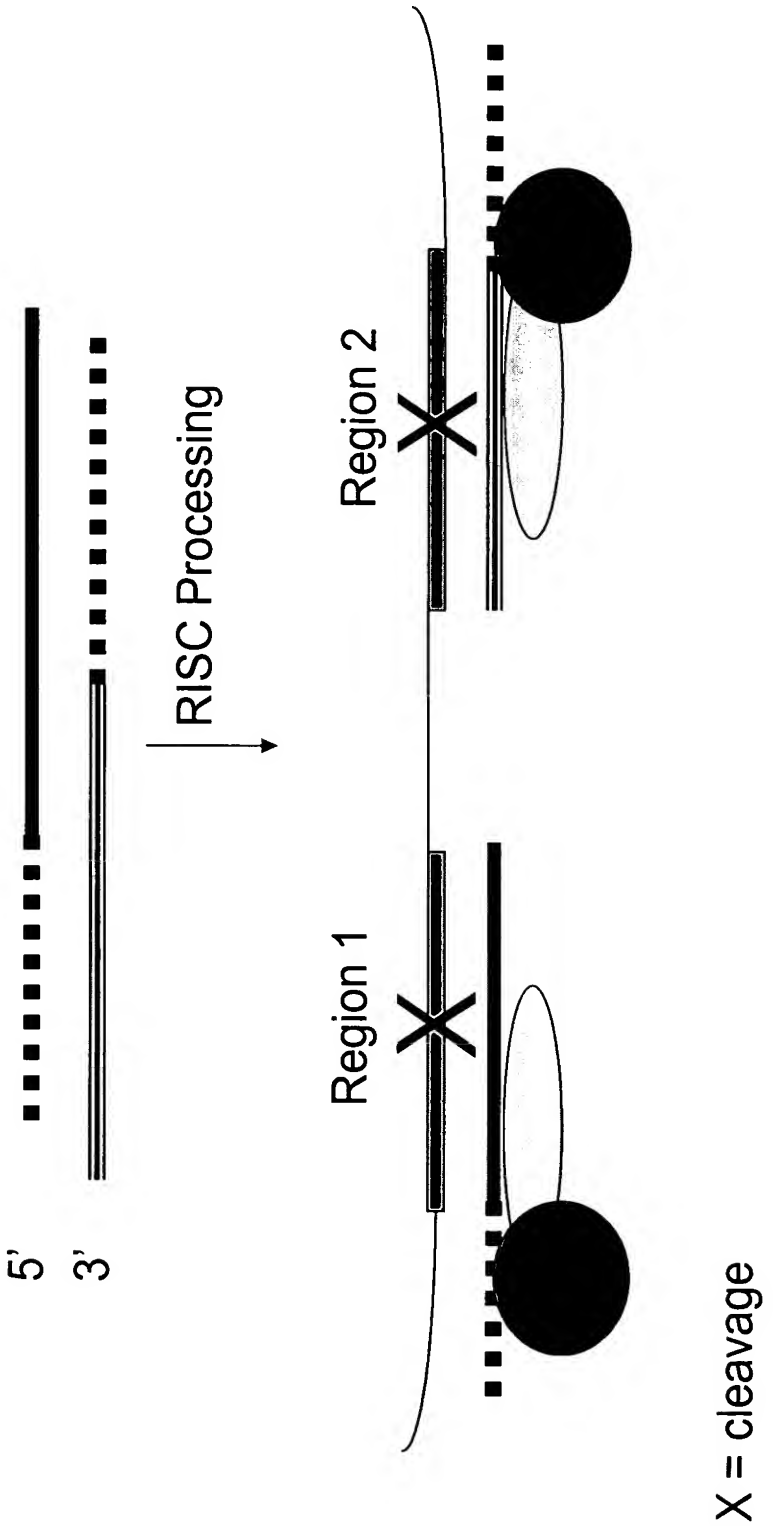


Figure 102: Activity of Systemically Administered Stabilized siNA in HBV Mouse Model: Serum HBV DNA Levels

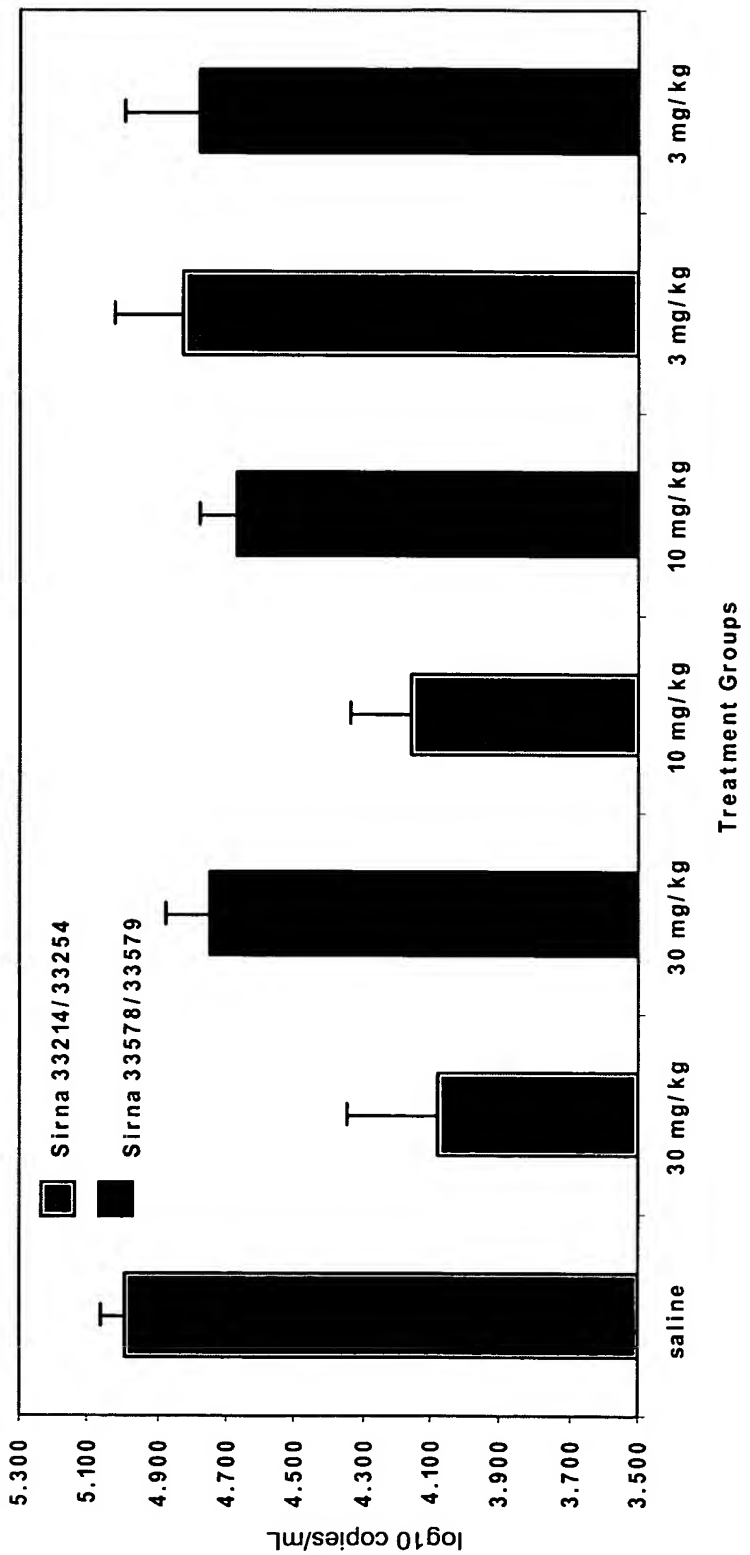


Figure 103A: Activity of Systemically Administered Stabilized siNA vs all RNA siNA in HBV Mouse Model: Serum HBV DNA Levels

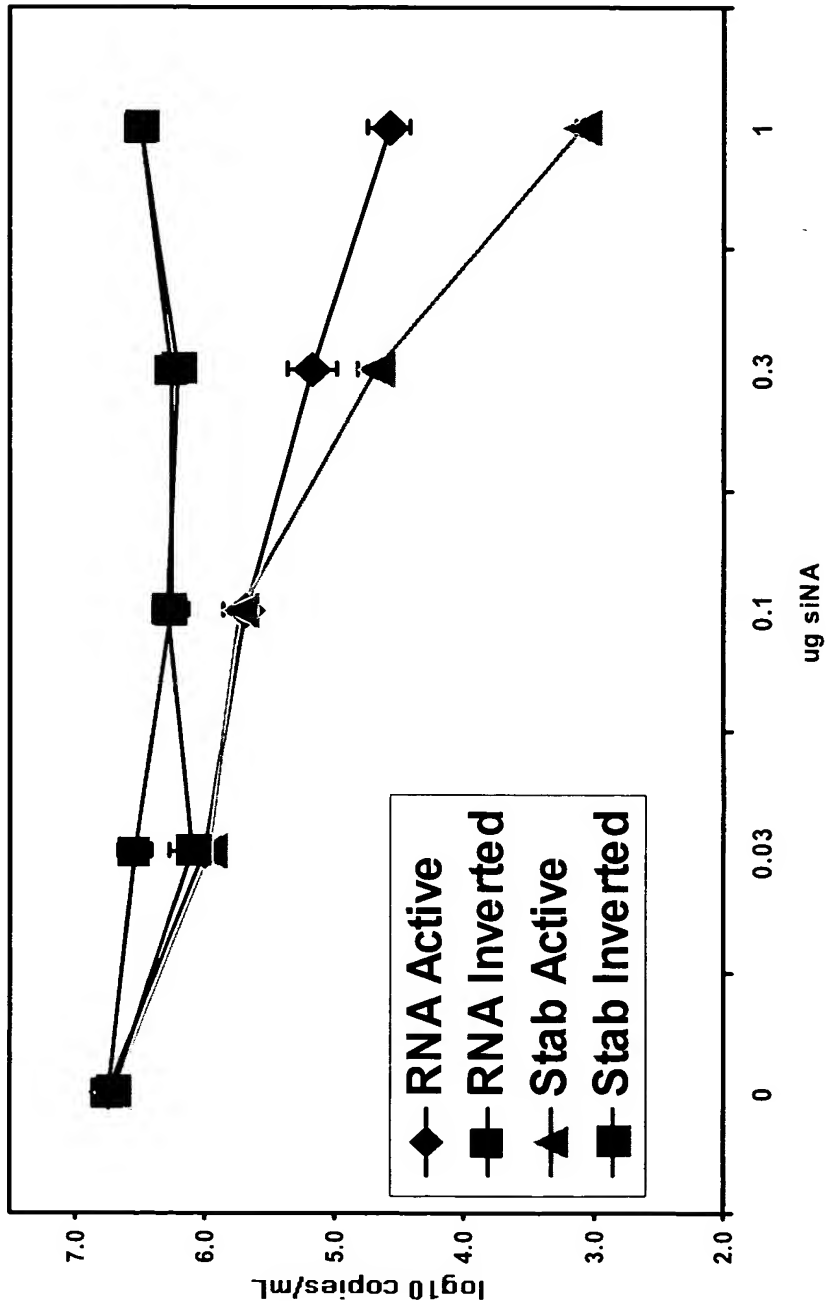


Figure 103B: Activity of Systemically Administered Stabilized siNA vs all RNA siNA in HBV Mouse Model: Serum HBsAg Levels

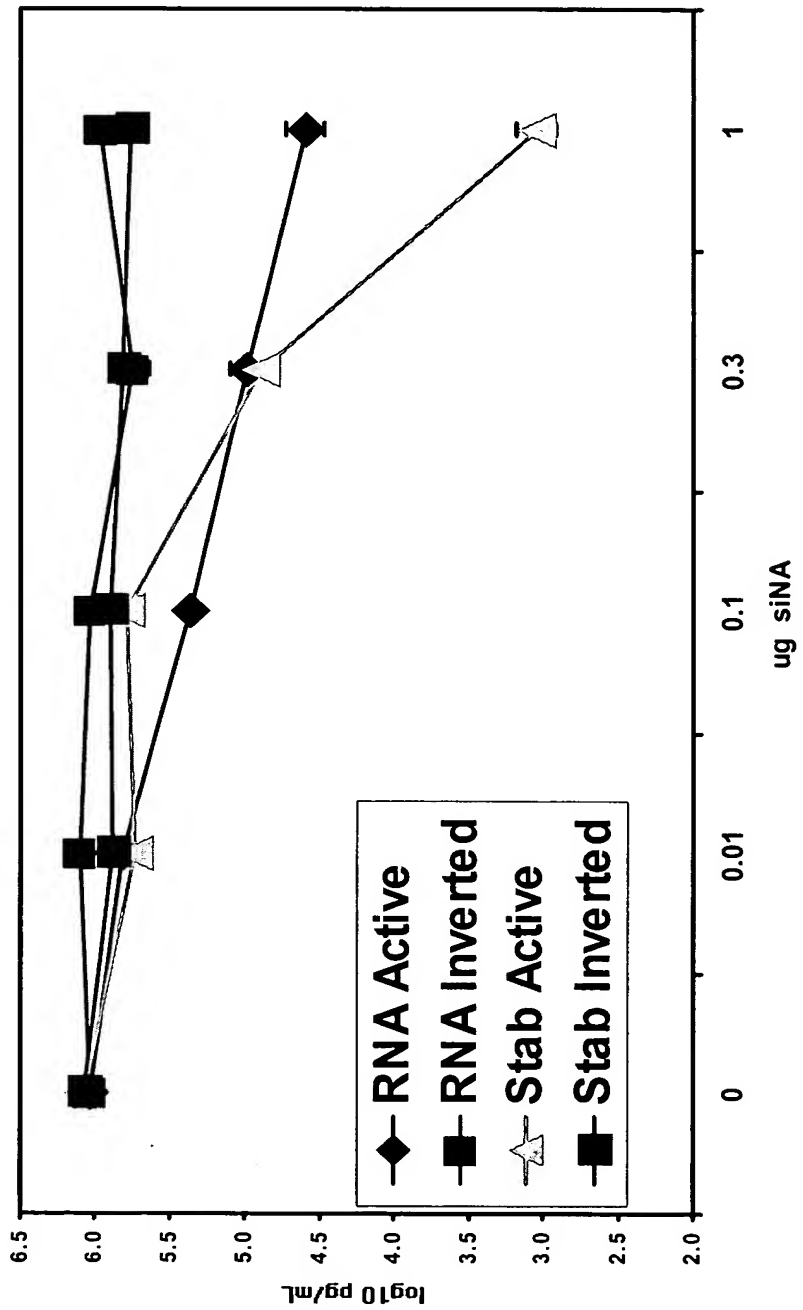


Figure 103C: Activity of Systemically Administered Stabilized siNA vs all RNA siNA in HBV Mouse Model: Liver HBV RNA Levels